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THE NUTRITION OF THE SPRING CABBAGE

BY R. M. WOODMAN
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(With Plate 9)

THIS communication is a continuation of investigations on vegetable nutrition. The experiments described were carried out on Sutton's Harbinger spring cabbage grown in sand under glass.

EXPERIMENTAL

Methods have been described previously. Glazed pots were used for the experiments with potash, phosphate, and boron, and waxed plant-pots for those with nitrogen (Woodman, 1941). Concentrations of certain elements present in the nutrient solutions are given in Table 1 as p.p.m. Variations from the control in the concentrations of the elements under experiment (N, P, K and B), and of the combined elements (Na and S), are italicized. The salts containing the elements have already been described (Woodman, 1939).

TABLE 1. *Concentration in p.p.m. of important elements in the culture solutions*

Solution	N	P	K	B	S	Na
			Nitrogen experiment			
A	*65·92*	21·84	22·44	0·0681	16·44	*140·7*
B	*65·92*	21·84	22·44	*Nil*	16·44	*140·7*
C	32·96	21·84	22·44	0·0681	16·44	86·56
D	*16·48*	21·84	22·44	0·0681	16·44	*59·48*
E	*8·24*	21·84	22·44	0·0681	16·44	*45·94*
F	*4·12*	21·84	22·44	0·0681	16·44	*39·17*
G	*0·41*	21·84	22·44	0·0681	16·44	*33·08*
H	*Nil*	21·84	22·44	0·0681	16·44	*32·40*
			Phosphorus experiment			
A	32·96	*43·68*	22·44	0·0681	16·44	*118·96*
B	32·96	21·84	22·44	0·0681	16·44	86·56
C	32·96	*10·92*	22·44	0·0681	16·44	*70·36*
D	32·96	*5·46*	22·44	0·0681	16·44	*62·26*
E	32·96	*2·73*	22·44	0·0681	16·44	*58·21*
F	32·96	*0·27*	22·44	0·0681	16·44	*54·57*
G	32·96	*Nil*	22·44	0·0681	16·44	*54·16*
			Potassium and boron experiment			
A	32·96	21·84	22·44	0·0681	16·44	86·56
B	32·96	21·84	22·44	*Nil*	16·44	86·56
C	32·96	21·84	*11·22*	0·0681	*11·84*	86·56
D	32·96	21·84	*5·61*	0·0681	*9·54*	86·56
E	32·96	21·84	*2·81*	0·0681	*8·39*	86·56
F	32·96	21·84	*0·28*	0·0681	*7·36*	86·56
G	32·96	21·84	*Nil*	0·0681	*7·24*	86·56

In addition, there were present in all the solutions, 1·01 p.p.m. of iron as ferrous sulphate, 5·05 p.p.m. of magnesium as the sulphate, and 9·03 p.p.m. of calcium as chloride; the sulphur of the first two is contained in the amounts quoted in the table for sulphur. Minor elements were present also in the following proportions: 0·1847 p.p.m. of manganese as the sulphate tetrahydrate; 0·0682 p.p.m. of zinc as the sulphate; 0·0095 p.p.m. of aluminium as ammonium alum; 0·0164 p.p.m. of the ammonium radicle, NH_4^+, partly as the alum above, and partly as ammonium sulphate; and 0·0153 p.p.m. of copper as the sulphate. The elements combined with these small amounts of the minor elements in the above salts have been neglected in compiling the table.

The numerical data obtained

The eleven values obtained for each culture have been enumerated (Woodman, 1940), and a description of a table similar to Tables 2–4, together with methods of interpretation of the results, have been given. The symbols S, SS, SSS, and NS indicate that differences between the results for the different treatments are significant at the 5 %, 1 %, or 0·1 % points, or are not significant, respectively, for the value under discussion. The cabbages were cut off at sand-level.

Observations and discussion of results

Nitrogen experiment

The number of pots was 128, in a randomized block of eight treatments, A–H, Table 1, replicated sixteen times. Seed was sown 21 Nov. 1938, and germinated by 28 Nov. Singling was 1 Dec. 1938, and the harvest 26 April 1939. The greenhouse temperature was 50–55° F. Each pot received a total of 17 l. of the appropriate culture solution in lots of 250 c.c. three times a week.

Plants that received A appeared to be the largest, but they suffered severely from marginal scorch. Early there was a tendency to irregularity in the green, and a somewhat mottled appearance resulted. The marginal scorch started on the oldest, lowest leaves, as a soft-feeling, darker green portion, which later became a crisp feeling (but not brittle), grey-brown, marginal belt, extending in about 1–2 cm. The remaining portions of the leaves then became yellow, but not scorched, and the affected leaves dropped off, the plants thus being much smaller than they would have been but for this scorch. Some of the plants also suffered generally on most of the leaves from grey-green necrotic spots about 0·5–1·5 cm. across. The roots of plants with A were found to be normal at harvest. Six cabbages were then hearted, and eight showed definite signs of hearting.

Solution B, identical with A except that it contained no boron, resulted in similar severe marginal scorch; but superimposed on this effect of excess nitrogen was a definite mottling due to a lighter green. No photographs of the mottling in this particular experiment were obtained, but Pl. 9, figs. 1 and 2, show the mottling due to lack of boron in a similar experiment carried out in glazed pots in 1937–8. Fig. 1 shows the leaf by transmitted sunlight, and Fig. 2 by reflected sunlight. There was browning and death of the inner shoots of some of the cultures and, with others, production of mis-shapen, thickened, inner leaves with a tendency to curl up to form saucer- or cup-like shapes, the new leaves later appearing to be merely distorted, thickened, flat stems. Some notion of how the leaves tend to curl out of the usual flat plane can be got from Pl. 9, fig. 3. By harvest, new leaves had developed, the thickened growths had disappeared, and five of the cabbages showed a slight tendency to heart up, while others had an abundant growth of loose leaves; the result was, therefore, a cabbage of good average size (B, tops, Table 2). This recovery was probably due to the fact that the wax on the plant-pots had become friable and permeable to water, so that a supply of boron was obtained from the unglazed pot itself. Cultures in Pl. 9, fig. 4, however, from the glazed pot experiment of 1937–8 previously mentioned, show blindness arising from lack of boron; the subject will be taken up again under the potassium experiment, where the yield in the absence of boron approximated more to the truth.

Solution C yielded normal plants with no scorch. There was a tendency at first to a purple tint on the extreme tips of the leaf serrations. The cabbages developed purple flushes, but these disappeared later and green cabbages resulted at harvest, when seven were hearted.

The roots were normal. D gave smaller cabbages with no scorch, one being hearted; the roots were normal. E, F, G, and H gave still smaller cabbages with no scorch, no signs of turning in, and with a tendency to purple flushes on the older leaves, which died with G and H; the roots were normal, but small with G and H.

Differences between the results with different treatments were significant for all the eleven values at the 0·1 % point (SSS, Table 2). There was a progressive decrease in size of the cabbages in passing from A to F, G, and H; thus A > C > D > E > F = G = H (B may be ignored, because of partial recovery). A somewhat similar order held for the whole plants, though the roots with C were the largest (C > A = B = D > E > F > G = H). The fresh yields thus demonstrated that the greater the supply of nitrogen (as nitrate), the greater was the

TABLE 2. *Summaries of results for nitrogen experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	H	Mean of all results	S.E.
Tops, FW	SSS	105·3	95·56	90·63	47·83	25·32	11·90	1·44	0·896	47·37	3·839
		A > C > D > E > F = G = H; A = B; B = C									
Roots, FW	SSS	13·19	12·63	16·98	13·10	9·97	6·14	0·210	0·136	9·045	0·8592
		C > A = B = D > E > F > G = H									
Whole plants, FW	SSS	118·5	108·2	107·6	60·93	35·28	18·04	1·650	1·033	56·41	3·268
		A > B = C > D > E > F > G = H									
Tops, DW	SSS	13·51	11·80	14·65	8·31	4·60	2·15	0·171	0·131	6·916	0·4024
		A = C > B > D > E > F > G = H									
Roots, DW	SSS	2·49	2·42	3·13	2·28	1·53	0·838	0·037	0·036	1·596	0·1832
		C > A = B = D > E > F > G = H									
Whole plants, DW	SSS	16·00	14·22	17·78	10·60	6·13	2·99	0·208	0·167	8·512	0·5301
		C > A > B > D > E > F > G = H									
Top/root, FW	SSS	8·41	8·43	5·69	4·09	2·72	2·06	7·31	7·03	5·717	0·4897
		A = B = G > C > D > F; A = B = G = H; C = H > E = F; H > D; D = E									
Top/root, DW	SSS	6·08	5·59	4·95	4·06	3·41	2·67	4·57	4·12	4·431	0·4083
		A = B = C > E = F; A = B > D = E = H; A > D = E = G = H; B = G > F; C = D = G = H > F									
Tops, % moisture	SSS	87·18	87·70	83·77	82·65	81·85	81·88	88·37	84·65	84·77	0·4584
		A = B = G > C = H > E = F; D = E = F; C = D; H > D									
Roots, % moisture	SSS	81·56	81·25	81·23	82·25	84·64	86·07	81·77	73·09	81·48	1·187
		A = B = C = D = E = G > H; F > A = B = C = D = G; E = F									
Whole plants, % moisture	SSS	86·51	86·97	83·42	82·54	82·63	83·37	87·49	83·15	84·51	0·4669
		A = B = G > C = D = E = F = H									

yield, a normal finding largely borne out by the dry yields, though C was superior here for roots and whole plants. The quality of the cabbages with C, moreover, was definitely the best, as those with A and B were unmarketable because of scorch, and those given by solutions containing less nitrogen than C itself were too small to be profitable. There was too little regularity in the differences between treatments with top/root ratios and percentage moistures to call for much comment, although there was possibly a bias in favour of these values being larger for the greatest quantities of nitrogen and the least (G), except for the moisture in the roots, where E and F were largest, and H the smallest.

Phosphorus experiment

Large glazed pots were used, holding 43 lb. of sand. There were seventy, arranged as ten replications of seven randomized treatments A–G, Table 1. The seed was sown on 21 Nov. 1938, germination was complete by the 28th, the seedlings were singled on 1 Dec. 1938, and the harvest was taken on

24 April 1939. The greenhouse was maintained at 50–55° F. Each pot received 26·6 l. of its solution in lots of 400 c.c. three times a week.

Throughout the experiment the largest cabbages were those receiving solution D. Thus, on 31 March 1939, before turning in interfered with the size, the linear dimensions (average greatest breadth, and average at right angles to this, in cm.) were: A, 27·8 × 26·1; B, 27·3 × 25·5; C, 27·8 × 26·1; D, 29·7 × 26·5; E, 28·0 × 25·7; F, 9·3 × 7·8; and G, 7·3 × 5·8. The big drop in size between E and F that followed on a succession of comparatively equal sizes for A–E (also shown up well in the summaries of results, Table 3) was similar to the effect of phosphorus on the turnip (Woodman, 1941).

Plants with F and G were slightly darker throughout most of the experiment, and there was some tendency to purple tints. Otherwise the behaviour with all treatments was the same, except for differences in size and turning in. At harvest, solutions A, B, C, D, and E

TABLE 3. *Summaries of results for phosphorus experiment. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	144·9	148·6	156·0	160·0	149·2	9·63	4·70	110·4	4·616
		B=C=D=E>F=G; A=B=C=E>F=G; D>A								
Roots, FW	SSS	27·99	29·54	29·83	30·94	30·86	1·12	0·771	21·58	3·139
		A=B=C=D=E>F=G								
Whole plants, FW	SSS	172·9	178·2	185·8	190·9	180·1	10·75	5·47	132·0	6·230
		A=B=C=D=E>F=G								
Tops, DW	SSS	20·02	20·98	22·34	23·54	22·71	1·299	0·730	15·95	0·7011
		C=D=E>A; D>A=B>F=G; B=C=E								
Roots, DW	SSS	4·94	5·53	5·58	6·61	5·10	0·223	0·158	4·019	0·8193
		A=B=C=D=E>F=G								
Whole plants, DW	SSS	24·96	26·51	27·92	30·15	27·81	1·522	0·889	19·97	1·281
		B=C=D=E>F=G; A=B=C=E>F=G; D>A								
Top/root, FW	NS	6·55	5·37	5·49	5·93	5·02	8·54	5·55	6·064	0·8299
Top/root, DW	NS	5·42	4·13	4·23	4·30	4·65	5·19	3·83	4·535	0·6292
Tops, % moisture	NS	86·13	85·84	85·59	85·43	84·84	86·63	85·60	85·72	0·4230
Roots, % moisture	S	83·20	81·30	81·22	78·58	83·28	75·50	71·94	79·29	2·730
		A=B=C=E>G; A=B=C=D=E=F; D=F=G								
Whole plants, % moisture	NS	85·68	85·10	84·43	84·18	84·60	85·02	82·67	84·53	0·7894

yielded marketable cabbages of which five with A, six with B, eight with C, seven with D, and nine with E, were hearted, and all the others showed definite signs of turning in; the roots were normal, with normal fibre. With F and G there were no indications of turning in, and the cabbages were small and immature, with a tendency for the oldest leaves to turn purple and die; the roots were small but fibrous. That a certain amount of phosphorus was present in the seed was indicated by the size of the plants with G, phosphorus absent (see Table 3 also).

Differences between the results for the various treatments for the fresh and dry yields were all significant at the 0·1 % level (SSS, Table 3). The differences for the two top/root ratios and two of the moisture contents were not significant (Table 3, NS), while the root moistures were significant only at the 5 % level (S).

The yields (Table 3) showed that differences in concentration of available phosphorus had little effect on the size of the cabbage until a low concentration (between E and F,

2·73 and 0·27 p.p.m. of P, respectively) was reached; there the diminution in yield was great. This behaviour is more reminiscent of the turnip (Woodman, 1941) than the lettuce (Woodman, 1940), which latter thrived on large supplies of phosphorus.

It cannot be stated definitely from the results that any of the treatments A to E was superior to any other; but there was a bias in favour of D, 5·46 p.p.m. of available P, and certainly D was superior to A (D > A, for both fresh and dried tops).

Potassium and boron experiments

There were seventy glazed pots in all, ten replications of seven randomized treatments. Solution B (Table 1) contained no boron, borax being omitted in making it up; the treatments A, C, D, E, F, and G were with solutions containing varying amounts of potassium, denoted in Table 1. The seed was sown 30 Nov. 1939, and germinated by 12 Dec.; the seedlings were singled 10 Jan. 1940. The harvest was on 21 May 1940. The greenhouse was at 50–55° F. Each culture received 30·4 l. of its appropriate solution in lots of 400 c.c. three times a week.

By 30 Jan. 1940 there was a slight tendency to mottling with B (lack of boron), so that the plants appeared to be a little lighter green than those with A, C, D, and E; cultures with F and G were much smaller, the cotyledons were scorched and dead, there was a slate- or grey-green wilting of parts of the second leaves, and a tendency to chlorosis of the whole plants. On 27 Feb., A gave healthy dark green plants. B, with no boron, resulted now in smaller plants of lighter green, with the older leaves curled out of the usual flat plane and also down at the sides; new leaves tended to curl along the major and minor axes, to give mis-shapen, crinkled leaves somewhat narrower than the normal leaf, the whole plant in consequence appearing thin, narrow, and meagre; finally, there was also a tendency to mottling. C and D yielded normal plants. E gave plants of lighter green, with grey- or slate-green marginal scorch, composed of soft-feeling tissue, along the edges of the oldest leaves, and extending in about 0·75 cm. The portions of leaf next to this scorch were light green or yellow; and the hold of the petiole was loosened, so that the leaf eventually dropped off and reduced the size of the culture. With F and G the effects of a deficiency of potassium were still worse, and leaf-drop was severe.

Solution A yielded good cabbages (Pl. 9, fig. 5), nine of which were turning in at harvest. The older leaves tended to be tinted a red-orange or pink-amber colour, and the next oldest, slate-purple; some of these dropped off in the normal course of events.

Lack of boron with solution B (Pl. 9, fig. 6) led to very characteristic plants: there was a tendency to a dull, soft-feeling, grey-green scorch of the leaves that later turned slate-grey, dry, and brittle to the touch. The young leaf buds turned brown and rotted, and there was necrotic tissue on, and sometimes longitudinal cracking of, the upper surface of the leaf petioles. The young leaves eventually curled along both axes so much that they balled up into irregular spheres with the back of the leaf—sage-green due to secretion of wax—on the outside (there were numerous minute spottings of normal green, due to lack of wax, on this sage-green background). The older leaves were small, narrow, and long, as opposed to the approximately circular leaves of the normal plant, and were hard and brittle, and mottled with sickly, amber-green (often nearly amber-white) patches to such an extent that the cultures appeared to be abnormally light in colour; there was a broad, brilliant, scarlet-mauve edging on most of the upper surfaces of the leaves, and patches of this colour on the underneath surfaces. New, solid-looking buds formed in the axils of the leaf petioles, and

the largest of these rotted and died. There were also small buds all up the stalk of the cabbage. Most of the plants wilted and died before the harvest, and none hearted up. The roots tended to rot and to be lacking in fibre.

Solution C yielded good cabbages of slightly lighter shade than those with A, with a similar but slighter tendency to purple tinting; three were turning in at harvest; there had been, however, some scorch and leaf-drop, but to an extent smaller than that described for D following. This solution, D, yielded still lighter coloured cabbages that would have been of good size but for serious marginal scorch and necrotic spots due to a deficiency of potassium, followed by limpness, withering, and final leaf-drop of most of the lower, older leaves. At harvest the marginal scorch was slate-grey, or sometimes biscuit-brown, with darker necrotic spots. No turning in was evident. Leaf-drop had been so serious that the stalks

TABLE 4. *Summaries of results for potassium and boron experiments. Weights in g.*

Description of data		Treatment mean for A	B	C	D	E	F	G	Mean of all results	S.E.
Tops, FW	SSS	106·1	2·744	84·14	54·30	27·91	0·174	0·156	39·37	4·332
		A > C > D > E > B = F = G								
Roots, FW	SSS	15·57	0·328	13·32	8·57	2·58	0·045	0·027	5·78	0·9145
		A = C > D > B = E = F = G								
Whole plants, FW	SSS	121·70	3·072	97·46	62·88	30·49	0·219	0·183	45·14	4·733
		A > C > D > E > B = F = G								
Tops, DW	SSS	16·85	0·580	12·56	7·50	3·59	0·023	0·017	5·87	0·7330
		A > C > D > E > B = F = G								
Roots, DW	SSS	4·82	0·057	3·46	2·56	0·809	0·003	0·003	1·67	0·2522
		A > C > D > E > F = G; D > B = E; B = F = G								
Whole plants, DW	SSS	21·67	0·638	16·02	10·06	4·399	0·026	0·021	7·55	0·9061
		A > C > D > E > B = F = G								
Top/root, FW	SSS	7·197	17·69	7·361	6·861	11·59	4·569	6·143	8·77	1·899
		B > A = C = D = F = G; B > E > F; A = C = D = E = G								
Top/root, DW	SS	3·549	33·73	3·887	3·301	5·418	8·858	6·270	9·29	5·303
		B > A = C = D = E = F = G								
Tops, % moisture	SS	84·16	75·93	85·11	86·19	87·20	82·83	87·39	84·13	2·083
		A = C = D = E = F = G > B								
Roots, % moisture	SSS	67·99	82·91	73·43	70·45	70·43	92·06	87·57	77·83	2·172
		F = G > A = C = D = E; F > B; B = G > A = C = D = E								
Whole plants, % moisture	SS	82·17	76·42	83·67	84·06	85·68	85·12	87·57	83·53	1·742
		C = D = E = F = G > B; G > A > B; A = C = D = E = F								

appeared to be abnormally long. The roots were normal, but smaller than with A. E, F, and G gave similar plants, but with severer symptoms of potassium deficiency, and similar but smaller roots. With E, only those inner leaves that would have turned in to form the heart (no hearting was evident with D, E, F, or G, however) were left, the encircling 'wreath' leaves all having died following the slate-grey or biscuit-brown scorch (Pl. 9, fig. 7). It was evident that this scorch, mainly, had reduced the size of these plants, and that otherwise they might have been approximately as large as cultures with A. Thus lack of potassium does not seem to cause lack of growth directly, but mainly indirectly by scorch of the foliage followed by death and leaf-drop, and by the diminution in growth that naturally follows this reduction in leaf surface.

Differences between the results for the different treatments were all significant at the 0·1% point (SSS, Table 4), except for the top/root ratios for dry weights, and the percentage

moisture contents in the tops and whole plants, where the significance was at the 1% level (SS). The fresh and dry matters of the tops (cabbages) and whole plants followed the same order, A > C > D > E > B = F = G, while similar relations held for the roots. Lack of boron resulted in plants statistically no larger than those with absence of potassium or great deficiency of it (B = F = G). Ignoring the results with lack of boron for the time being, the order for all six values for fresh and dried matter was A > C > D > E > F = G, except for the fresh roots, where the only differences from this order are that A = C, and E = F = G. It is evident from this order that the cabbage plant, especially the top, responds well to available potassium, and that larger amounts than that contained in solution A might possibly be beneficial to growth: that is, that even solution A is not the optimum solution possible.

In general the two top/root ratios were statistically equal except for the treatment with lack of boron, B, when high ratios resulted. These high ratios indicated that the root suffered relatively more than the top from lack of boron. The moisture contents of the tops and whole plants were low with absence of boron. With the roots the moisture content was comparatively high, but this may have been due to the fact that the roots were often rotten and sodden through, and could not be wiped quite dry on washing previous to weighing.

Summary

The effects on spring cabbage of deficiencies of available nitrogen, phosphorus, and potassium, and of absence of boron, were investigated, and yields statistically examined. Diminished yields and later maturity followed from diminution in nitrogen, though excess of nitrogen made the cabbages unmarketable because of severe marginal scorch. Reduction in phosphorus caused little difference in yield or maturity until a low concentration was reached, when a sudden drop in yield resulted, behaviour more reminiscent of the turnip than the leaf-crop lettuce. Deficiency of available potassium caused severe marginal scorch, necrotic spots, a leaf-drop that caused diminished yield, and immaturity. Absence of boron caused a sickly, meagre-looking, amber-green coloured plant with narrow leaves with a relatively broad upper edging of brilliant scarlet-mauve and patches of this tint on the undersides; there was a soft-feeling, dull, grey-green scorch of the leaves that later became dry, brittle, and slate-grey; the young leaf buds turned brown and rotted; there was necrotic tissue on, and sometimes longitudinal cracking of, the upper side of the leaf petioles; there were buds in the axils and up the stalk, the former of which rotted; and the young leaves tended to curl and eventually ball up into irregular spheres with the underside (sage-green from secretion of wax, spotted by normal green where wax was missing in minute areas) outermost. The plants died early.

I thank Mr T. W. McKean and Mr J. N. Leonard for their help in these experiments, and Dr S. O. S. Dark for certain of the photographs.

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EXPLANATION OF PLATE 9

Fig. 1. Mottling of the leaf due to absence of boron, photographed by transmitted sunlight on 25 Feb. 1938.

Fig. 2. Mottling of the leaf due to absence of boron, photographed by reflected sunlight on 25 Feb. 1938.

Fig. 3. Curling of the leaves due to absence of boron. Pot A received boron (solution A, nitrogen experiment, Table 1); pot B received the same quantities of nutrients except for absence of boron (solution B, nitrogen experiment, Table 1). Photographed 17 Feb. 1939.

Fig. 4. Blindness due to lack of boron. Photographed 23 March 1938.

Fig. 5. A normal spring cabbage grown with solution A, potassium and boron experiment, Table 1. The indicator divisions are inches. Photographed 19 March 1940.

Fig. 6. Effect of absence of boron. This culture received solution B, potassium and boron experiment, Table 1, identical with solution A (which gave the plant shown as Fig. 5) except for absence of boron. Note the balling of the new leaf, the small, relatively long, older leaves, and the generally tall and meagre appearance. Photographed 19 March 1940.

Fig. 7. Results of a deficiency of potassium (solution E, potassium and boron experiment, Table 1). Note the large plant, despite this deficiency. Two of the oldest leaves are dead on the sand, and a third one, facing the observer, is wilting and dying, and shows (slate-grey) marginal scorch extending inwards. Spots of (grey-green) scorch can be seen on the other leaves. Photographed 19 March 1940.

(*Received* 21 *February* 1941)

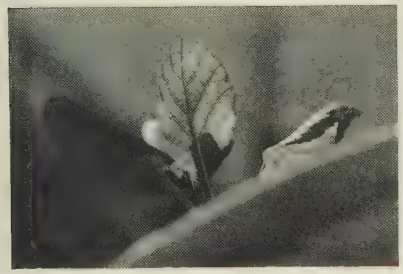

Fig. 1.

Fig. 2.

A B

Fig. 3.

Fig. 7.

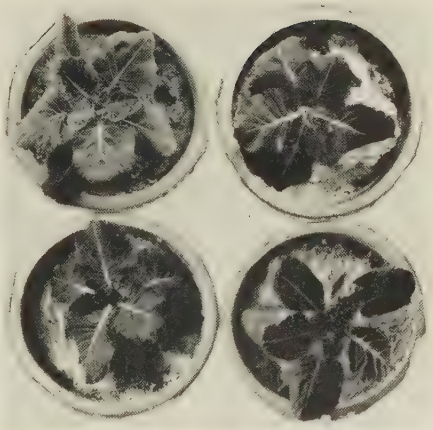

Fig. 4.

Fig. 5.

Fig. 6.

WOODMAN.—The Nutrition of the Spring Cabbage (pp. 181–88)

THE INFLUENCE OF LITHIUM SALTS ON CERTAIN CULTIVATED PLANTS AND THEIR PARASITIC DISEASES

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(With 3 Text-figures)

IIUM is known to act upon cultivated plants in three principal ways: it increases the tance to disease, in small quantities it stimulates growth, and in large quantities it bits growth and is toxic. There is, however, no information to show how these changes nduced, and the proposed theories cannot be substantiated or disproved until the con- ration and distribution of lithium in the plant are known: this information is not lable.

1 these experiments plants grown in soil culture were analysed for lithium, and measure- its were made of the alterations in the amount of growth and the susceptibility to disease sed by applications of lithium; in this way it was hoped to throw some light upon the hanism of the lithium effects. The plants used were:

(i) Celery (*Apium graveolens*) with leaf spot (*Septoria Apii* Chester).
ii) Wheat (*Triticum vulgare*) with powdery mildew (*Erysiphe graminis* DC.).
iii) Wheat (*Triticum vulgare*) with brown rust (*Puccinia triticina* Erikss.).
iv) Tomato (*Lycopersicum esculentum*) with crown gall (*Bacterium tumefaciens* E. F. Sm. Towns.).

A review of published work concerning the effect of lithium on the growth of higher and er plants and on susceptibility of higher plants to disease is in the press (Wortley, 1941). hium is widely distributed (Robinson, 1914), occurring in most soils in small quantities einkoenig, 1915), and was present in the soil used in these experiments. The presence of ium in plants depends on two factors: its occurrence in the soil, and the absence in the nt of any mechanism to prevent its absorption. The amount of lithium absorbed from thium-containing soil varies with the species (Headden, 1921); some plants (e.g. tobacco l many of the thistles) apparently have no means of preventing its entry, whereas closely lated plants take up very little.

Materials and methods

Culture of the host. The plants were grown in a small greenhouse which was heated in winter. Seeds celery (Wright's Grove Red and Clayworth no. 1), wheat (Red Marvel and vulgare p.p.) and tomato ondine Red) were sown in boxes or pots containing good potting soil, leaf mould, sand and peat in proportion 5:2:1:1. The celery seedlings were pricked out at the one or two leaf stage into 3 l. boxes, with forty plants (four rows of ten) per box. The wheat seedlings were not transplanted but thinned to about eight seedlings per 4 in. pot. The tomato seedlings were pricked out separately at the first leaf stage into 4 in. pots containing a soil mixture with a high proportion of leaf mould and a little lime. They were repotted successively into 5 and 7 in. pots as soon as they showed signs of becoming pot-bound; all the plants in any one experiment were repotted at the same time.

Lithium application. Lithium was applied in solution with a pipette to the surface of the soil between the plants; care was taken to prevent the solution splashing them. The salts used, lithium chloride (LiCl) and lithium nitrate ($LiNO_3 3H_2O$), were made up in 5 % solution for stock and diluted as required. The 3 l. boxes received 40 c.c., the 4 in. pots 10 c.c.; with these volumes the soil surface could be fairly uniformly wetted but the liquid did not drain through. The amount of lithium applied

was varied either by using solutions of different strengths and applying the same volume to each or by using only one solution and applying successively repeated doses to increase the total quantity supplied. The latter method was more satisfactory because the toxic effect of lithium on the plants was minimized but the effect on the disease was not reduced. Celery and tomato were treated when three or four leaves were present, wheat at the one-leaf stage. In the tomato experiments some of the lithium added to the soil was soaked up by the earthenware pots and disastrously affected other plants subsequently grown in them. To overcome this difficulty the 4 in. pots used in later experiments were waterproofed by soaking in melted paraffin wax. This treatment was not extended to the 5 and 7 in. pots partly on account of the difficulties involved in processing such large pots and partly because no lithium was applied after the 4 in. pot stage. The lithium concentration was expressed as milligram-equivalents of lithium per litre of soil. This method of expression assumed a uniform packing of the soil in the various boxes but did not necessitate weighing the soil or determining its degree of wetness; it enabled the effect of chloride and nitrate to be compared correctly and it took into account the depth of the soil in the boxes.

Culture of the pathogen. The original cultures of *Septoria Apii* and *Bacterium tumefaciens* were obtained from the University Botany School, Cambridge, and were maintained on tube slopes of 1·5 % malt agar; from these, inoculum was obtained for celery leaf spot and tomato crown gall. Leaf spot inoculum was also obtained from diseased celery leaves. Cultures of *Erysiphe graminis* and *Puccinia triticina* were maintained on wheat plants kept separately from those used in the experiments.

Inoculation and disease estimation. Celery was inoculated with a spore suspension applied with an atomizer, using equal volumes of suspension for each box of plants. The plants were placed for 2 days in zinc glass-topped inoculation tanks, within which a high humidity was maintained, and thereafter placed on the greenhouse staging. The interval between lithium treatment and inoculation was varied in different experiments. Estimations of the amount of disease present were begun as soon as pycnidia appeared. Counts of the number of pustules per box of plants were made usually at 2-day intervals from the outbreak until either the pustules had merged together so as to make accurate counting impossible or until the oldest leaves had withered. In some experiments the infection was assessed for each individual leaf in order to separate primary infection from secondary and to relate susceptibility to leaf age. The efficiency of various lithium treatments in reducing susceptibility to leaf spot was expressed by dividing the figure for disease on the control plants by the corresponding figure for the lithiated plants. This ratio (the C/L ratio) was found to fluctuate during development of the disease, rising to a maximum at about 18 days from inoculation and thereafter decreasing. The amount of disease was also expressed as the relative disease intensity, i.e. the number of disease pustules on the treated plants relative to those on the corresponding untreated plants, the figure for which was set at 100.

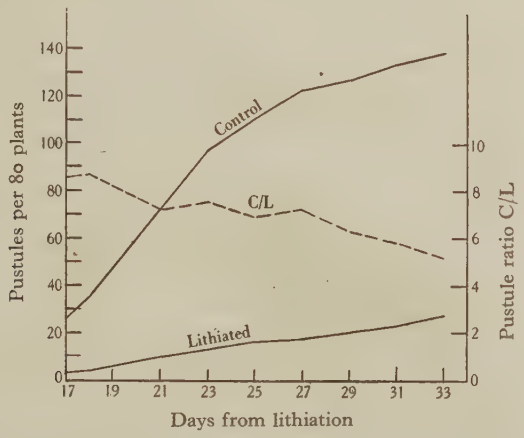
Wheat was inoculated with mildew a few days after lithium treatment: by transference of spores from mildewed plants in winter and by merely placing the pots near to mildewed plants in the summer. The disease intensity was estimated by observing the amount of mycelium and spores visible on the leaf; each leaf was assessed at a value from 0 to 4, and the mean intensity found for each treatment. For comparison the disease intensity on the control plants was taken as 100 and that on the treated plants expressed relative to it. Wheat was inoculated with brown rust by drawing the leaves between the moistened fingers, removing the waxy bloom and leaving a film of moisture (Mains & Jackson, 1926) and applying uredospores in a water suspension with a small brush to their upper surfaces (Hungerford & Owens, 1923). The disease intensity was estimated by counting the number of pustules on a measured length and expressing the result as pustules per cm. length of diseased leaf. Only areas of leaf thickly covered with pustules were used.

Tomato plants were inoculated with a suspension of bacteria in water obtained by scraping the bacteria off the agar and floating them on water in a watch-glass. Young cultures were always used as a source of inoculum. The suspension was left for ½ hr. until the bacteria had become evenly diffused throughout the water, giving an opalescent solution. The bacteria were introduced into the stem by means of a sterilized needle. In some experiments a single prick was made through one node of each plant, producing a single pair of galls; in others, a series of five nodes was inoculated on each plant producing five pairs of galls. The plants usually received the first lithium application a day or two before inoculation. The galls developed slowly and the plants were grown on for 3–4 months. Three methods of measuring gall size were used. The gall size was judged *visually* and the plants classified into numbered categories; and 'index of intensity' was then assigned to each treatment. The *diameter* of the stem and gall pair was measured with dividers and also the width of the stem alone just above and just below the gall. The mean stem width was subtracted from the first measurement, giving the

net gall width. The *weight* of the galls was obtained in two ways: the gall was cut off flush with the stem and weighed; alternatively, 1 in. length of stem, including the gall, was cut with a tool consisting of two razor blades clamped 1 in. apart; adjacent 1 in. lengths of the stem from above and below the gall were also cut and their mean weight subtracted from the weight of the first piece, giving the net gall weight.

Estimation of host-plant yield. Celery and wheat were not grown to maturity but were weighed as soon as possible after the last estimation of disease intensity. The separate tops and roots were collected in tared tins and weighed fresh, dried and ashed; in some experiments the leaves were collected chronologically and divided into laminae and petioles for separate weighing. The fresh-weight yield of the tops and roots of tomato plants was measured when the galls were being weighed.

Lithium content of the host plants. Lithium in the ash of celery and wheat and of tomato galls was estimated spectrographically by the Ramage flame method (Kent, 1940). The percentage lithium uptake was estimated by relating the corrected total quantity found in the plants to that supplied; the 'correction' made allowance for the small amount of lithium invariably found in the control plants derived from the untreated soil: it involved subtracting the figure for the lithium content of the control plants from that of the treated plants.



Text-fig. 1. The progress of leaf spot disease on lithiated and control celery plants as measured by pustule count (continuous lines). The broken line is the ratio control/lithiated. Dose: 4·8 mg.-equiv. Li/l. soil.

Celery and leaf spot

Progress of the disease. The relative rate of disease increase was estimated by making successive counts of the number of pustules. The incubation time between inoculation and the appearance of pustules varied according to the temperature and humidity of the season (e.g. 21 days in midwinter, 11 days in midsummer) and with the inoculum; with spores from leaves the disease appeared sooner than with spores from tube cultures. The primary disease produced by the initial inoculation could infect younger leaves producing secondary infection after a further incubation period. The secondary infection was invariably much denser than the primary. A curve plotted between the number of primary infection pustules and time from inoculation was roughly S-shaped (see Fig. 1).

Effect of varying the lithium concentration. The results obtained when lithium nitrate and chloride were applied in varying concentrations (other conditions being constant) are summarized in Tables 1 and 2. The highest concentrations were used only in preliminary experiments in which the plant ash was not analysed for lithium. Effects of nitrate and chloride were similar; all tested concentrations reduced the relative disease intensity, and

more effectively as the concentration was increased. Fresh and dry weights were increased by small concentrations but were decreased by large ones. Within the range of concentrations chosen for later experiments (those which produced reduction in disease but no decrease in yield) both the total lithium content of the tops and the lithium concentration in the laminae were considerably increased by the treatment; highest concentrations were found in the laminae (see Table 2). Where the effects of equivalent doses of lithium nitrate and lithium chloride were directly compared the nitrate was found always to be slightly more efficient both for reducing disease and increasing the yield; the percentage uptake of lithium was larger from the nitrate.

TABLE 1. *Effect of lithium nitrate upon late blight disease, yield and lithium content of celery*

Li applied: mg.-eq./l. soil	Rel. disease intensity		Rel. wt. (whole plant)		Li content (whole plant)	Li conc. (mg. Li/100 g. fresh material)	
	Pustules	Plants	Fresh	Dry	μg./pl.	Tops	Roots
0·00	100	100	100	100	6·15 (5)	0·59 (3)	0·26 (30)
0·13	—	99 (1)	106 (1)	98 (1)	—	—	—
0·27	99 (1)	99 (1)	124 (2)	134 (2)	—	—	—
0·53	80 (1)	87 (3)	123 (3)	122 (3)	—	—	—
1·08	60 (1)	—	129 (2)	129 (2)	44·0 (1)	6·1 (1)	1·62 (1)
1·62	58 (2)	88 (2)	105 (1)	110 (1)	187·0 (2)	12·4 (2)	3·43 (1)
2·00	42 (3)	84 (1)	—	—	—	—	—
2·16	37 (1)	—	197 (1)	178 (1)	108·5 (1)	13·7 (1)	1·12 (1)
3·24	41 (2)	75 (1)	130 (3)	131 (2)	231·6 (2)	15·1 (2)	3·25 (1)
5·3	—	—	101 (1)	96 (1)	—	—	—
8·1	—	—	23 (1)	39 (1)	—	—	—

Each value is the mean of the number of experiments given in parenthesis; this applies to all the tables.

TABLE 2. *Effect of lithium chloride upon late blight disease, yield and lithium content of celery*

Li applied: mg.-eq./l. soil	Rel. disease intensity		Rel. wt. (whole plant)		Li content (whole plant)	Li conc. (mg. Li/100 g. fresh material)		
	Pustules	Plants	Fresh	Dry	μg./pl.	Laminae	Petioles	Roots
0·00	100	100	100	100	6·15 (5)	1·04 (3)	0·09 (2)	0·26 (3)
1·62	67 (6)	85 (4)	111 (7)	100 (6)	108·5 (1)	19·1 (1)	1·02 (1)	1·59 (1)
2·35	62 (1)	—	—	—	—	—	—	—
3·24	37 (5)	74 (9)	106 (11)	98 (9)	204·0 (4)	20·2 (5)	1·05 (4)	5·74 (3)
4·80	*14·5 (4)	51 (5)	104 (8)	97 (7)	—	—	—	—
7·75	—	—	70 (1)	90 (1)	—	—	—	—
11·75	—	5 (1)	37 (1)	48 (1)	—	—	—	—
15·5	—	—	29 (1)	52 (1)	—	—	—	—

* If secondary infection is included the figure is 35 %.

Effect on primary and secondary disease. Lithium treatment had least influence on disease on the cotyledons; its greatest effect was upon secondary disease on the leaves. The disease on plants 36 days after treatment with 4·8 mg.-equiv. LiCl/l. of soil was as follows: on cotyledons 57 %, primary disease on leaves 40 %, secondary disease 1·4 % of that on the control plants. Reduction of disease due to lithium was greatest on the fifth and sixth leaves; these together bore only 10 % of the total disease on the lithiated plants whereas the same leaves on the untreated plants bore 23 % of the total.

Form of application. The gradual decrease of the C/L ratio for pustules (see above, p. 190) showed that the effect of a single lithium application was transitory and was related to the

lithium uptake by the plant; by prolonging the period of uptake, i.e. by giving numerous small applications over an extended period, it was hoped that the effect on the disease might persist (shown by a more constant C/L ratio). When a total of 4·8 mg.-equiv. LiCl/l. soil was given in three or six equal portions at intervals of a few days the C/L ratio remained constant for about 8 days, showing that a prolonged uptake of lithium had a more persistent effect on the disease; this type of staggered application was less toxic to the celery.

Variation in time intervals. The efficiency of lithium in reducing celery leaf spot increased as lithium application preceded inoculation by longer times up to about 12 days. Lithiation following inoculation (i.e. during incubation) was least effective. This result can be explained by assuming that disease reduction depends on the plant's lithium content (evidence is given below that this is a true assumption); a longer lithiation-inoculation interval gave more time for absorption of lithium. The greatest lithium content was found in the largest leaves whose size had been increasing rapidly (leaves of medium age; see below, Table 4). It is possible that the ascending lithium stream had been diverted after 12 days to younger leaves whose growth rates and final size had surpassed those of the slightly older leaves which first accumulated the lithium. The efficiency of lithium would therefore not increase indefinitely, as lithiation preceded inoculation by more than a certain optimum time.

The stimulative effect of small quantities of lithium on the yield of celery persisted and became more marked as long as the lithium content remained small. When a large amount of lithium had been accumulated, the growth was retarded as it is by a large dose. When most of the applied lithium had been absorbed and inactivated by deposition in the older leaves, the growth of the plants reverted to normal. Yield was measured at various times from an application of 1·62 mg.-equiv. LiCl/l. soil. At 57 days (the duration of the experiment) the relative fresh weight was 150 %, the relative dry weight 138 %; but after only 29 days from treatment the relative yield of the treated plants was less than 100, i.e. a toxic effect accompanied the absorption of quantities smaller than those which caused stimulation. A growth curve showing a similar 'double minimum' is given by Brenchley (1932) in her work on lithium, and Lundegårdh (1931) mentions that the growth curve obtained when increasing quantities of heavy metals were added to the nutrient solution is apparently periodic in shape.

Effect of lithium on yield of parts of the plant. The toxic effect of large applications was more marked on the roots, whereas the stimulative effect of small applications was shown more in the increased growth of the tops. Thus all applications of lithium tended to increase the tops/roots weight ratio. Lithium treatment increased the water content of the fresh material; this was apparently due rather to an increase in water uptake than to a reduction of transpiration, since the yield of the treated plants was larger.

Distribution of lithium in the plant. Figures showing the distribution of lithium among the various parts of the treated and untreated plants are given in Table 3. The laminae contained most, the roots considerably less and the petioles only a minute amount (the petioles comprise about 40 % of the tops by fresh weight, 30 % by dry weight). The percentage of lithium in the tops was increased by lithium treatment. The lithium content of various parts of celery plants, 27 days after treatment with 3·2 mg.-equiv. LiCl/l. soil, is given in Table 4, where the lithium concentration in the same parts is also recorded. The maximum laminar concentration was found in the fifth leaf (i.e. the largest leaf), while the maximum petiolar concentration was in the oldest leaf.

Concentration of lithium in the leaf tissues. The distribution of lithium in the lamina was investigated by dissecting it and analysing each part separately. The lamina was divided into four fractions—margin, lower and upper epidermis and the remainder. A description of the 'margin' and full details of the method of dissection have been published elsewhere (Kent, 1940). The lithium concentration in these parts is recorded in Table 5, where figures

TABLE 3. *Distribution of lithium in young celery plants*

Li salt used	Conc. applied: mg.-equiv./l. soil	% of total Li in Laminae	Petioles	Tops	Roots
Nitrate	0·00	74·5 (2)	3·0 (2)	77·5 (4)	22·5 (4)
,,	1·08	—	—	79·0 (1)	21·0 (1)
,,	1·62	86·5 (1)	2·0 (1)	88·5 (1)	11·5 (1)
,,	2·16	—	—	91·5 (1)	8·5 (1)
,,	3·24	—	—	95·0 (2)	5·2 (2)
Chloride	0·00	74·5 (2)	3·0 (2)	77·5 (4)	22·5 (4)
,,	1·62	87·0 (1)	3·0 (1)	90·0 (1)	10·0 (1)
,,	3·24	78·6 (3)	5·1 (3)	83·7 (4)	16·3 (4)

TABLE 4. *Total lithium content of laminae and petioles; lithium concentration in laminae and petioles*

Leaf no. (oldest)	Li content: μg. per plant Laminae	Petioles	Whole leaf	Li conc.: mg./100 g. Fresh Laminae	Petioles	Ash Laminae	Petioles
1, 2, 3	8·0	2·7	10·7	—*	—*	155	150·0
4	24·7	2·4	27·1	14·4	1·32	480	65·0
5	59·8	2·6	62·4	15·2	0·56	630	32·5
6	22·8	2·5	25·3	7·5	0·49	280	31·5
7	8·3	1·5	9·8	5·0	0·50	270	31·2
8	1·1	0·4	1·5	2·4	0·43	130	23·7
9 (youngest)	0·4	0·5	0·9	0·7	0·53	28·5	19·5

* The fresh concentration values for the fraction 1–3 are abnormally large because the leaves were withered when collected and the fresh weights recorded were not a true record of their weight when alive and healthy.

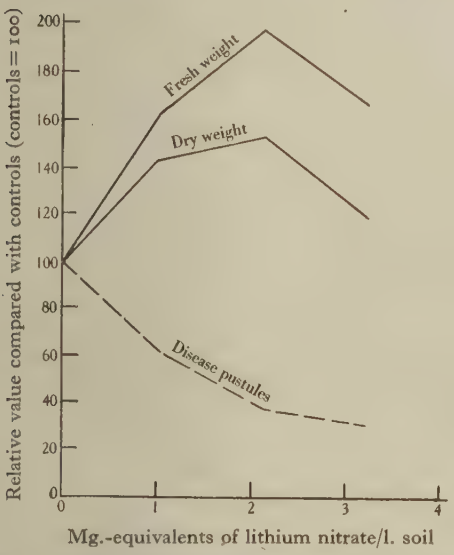
TABLE 5. *Lithium concentration in the ash of various parts of the lamina of celery plants*

Part of plant	Conc. of Li applied: mg.-equiv./l. of soil 1·62 nitrate	3·24 chloride
Whole lamina	2·6	4·75
Petiole	0·2	0·23
Lamina margin	7·3	13·5
Lower epidermis	1·45	2·5
Upper epidermis	1·5	2·85
Remainder of lamina	1·75	

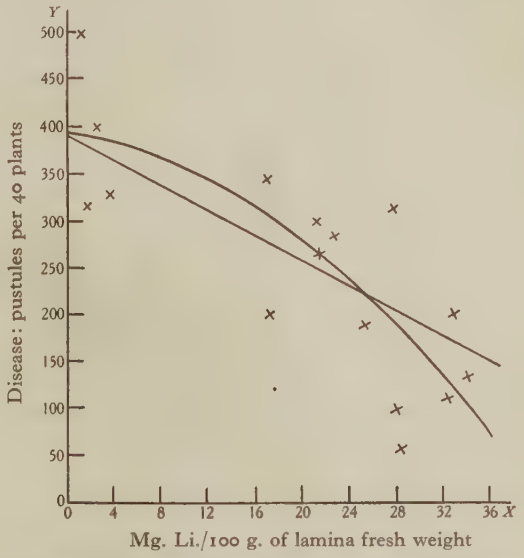
for the concentration in petioles and the whole lamina are also given for comparison. The concentration in the margin was always considerably larger than that in the whole lamina; it was calculated in one experiment that about 80 % of the total lithium in the lamina was situated in the 'margin'. The percentage of the total disease pustules which occurred on the 'marginal' area was small; it was considerably smaller for lithium-treated than for control

plants, suggesting that within the one leaf there was a differential effect of lithium in decreasing susceptibility to leaf spot according to the concentration in the various parts of the leaf.

Lithium content at various times from lithiation. In one experiment samples were taken for analysis 7 days after treatment with 3·2 mg.-equiv. LiCl/l. soil and thereafter at 5-day intervals up to 62 days. The total lithium content of both tops and roots continued to increase up to 57 days. The concentration in the tops, on the other hand, remained after 20 days at about 2·7 mg. Li/100 g. ash, whereas the concentration in the roots progressively decreased after 20 days to the end of the experiment (62 days). That is to say, root growth outstripped lithium absorption; by 62 days, 20 % of the added lithium had been removed by the plants. The coefficient of correlation between lithium content of the whole plant and time since lithiation was $+0{\cdot}91$ ($n=10$, $P<0{\cdot}01$).



Text-fig. 2. Effect of lithium nitrate upon the relative yield of celery (continuous line) and the relative intensity of leaf spot (broken line).



Text-fig. 3. Scatter diagram showing relation between lithium content of celery laminae and intensity o leaf spot. Equations of the line and curve are given in the text (p. 196).

Leaf spot disease and yield of celery plants. A graph plotted between the concentration of lithium applied and the relative disease intensity (see Tables 1 and 2) falls fairly steadily from the value for the controls, whereas a graph between the concentration of lithium applied and the relative yield shows an initial rise followed by a fall. These curves are shown in Fig. 2 for a typical experiment; yield and infection show apparently little relation to one another. The graph can be separated vertically into two parts at the figure for maximum yield. On the left of this line the correlation between yield and disease is inverse, on the right it is direct. The inverse relationship can be illustrated by comparing the yield of inoculated and uninoculated plants (untreated with lithium). The results recorded in Table 6 show that the weight ratio drops as the density of disease pustules increases. This is to be expected, since the disease causes the leaves to shrivel and eventually to drop off, reducing

the leaf area available for photosynthesis. The direct relationship between yield and disease, which obtains with higher concentrations of lithium (right-hand side of Fig. 2), is to be explained by assuming that at the turning-point of the yield graph the lithium first becomes toxic, and that as the concentration increases both toxicity to the host plant and the inhibiting effect on the fungus become more marked. The important result of these experiments is to show that stimulation of host-plant growth and reduction of susceptibility to disease can occur simultaneously; their coincidence depends upon choosing the correct lithium treatment. Smaller concentrations did not reduce susceptibility, while larger doses were toxic and reduced the yield.

TABLE 6. *Effect of leaf spot disease on the yield of celery plants*

Mean no. of pustules per 40 plants at the last count	Ratio $\frac{\text{inoculated}}{\text{uninoculated}} \times 100$	
	Fresh wt. tops	Dry wt. tops
32	101	113
36	104	112
42	97	98
63	85	93
91	89	91
108	92	94
342	75	85
569	63	76
803	57	54

Lithium in the leaf and leaf spot disease. It has been shown that reduction of relative disease intensity by lithium treatment was accompanied by increased lithium concentration in the leaf. The coefficient of correlation between lithium concentration in the laminae and disease intensity was $-0{\cdot}791$ ($n=14$, $P<0{\cdot}01$) in an experiment where there were sixteen pairs of values. The partial correlation between lithium concentration and disease intensity allowing for intercorrelation with lamina yield was $-0{\cdot}877$ ($n=13$, $P<0{\cdot}01$). The lithium concentration figures for the laminae were used rather than the mean figure for the plant tops as a whole, since the disease occurs almost exclusively on the laminae. Equations expressing the regression of disease on the lithium concentration in the laminae were as follows:

$$Y' = 391{\cdot}77 - 7{\cdot}0476X, \qquad \Sigma(Y - Y')^2 = 99{,}177{\cdot}9, \tag{i}$$

$$Y'' = 394{\cdot}4 - 2{\cdot}08X - 0{\cdot}188X^2, \quad \Sigma(Y - Y'')^2 = 58{,}161{\cdot}1, \tag{ii}$$

where Y is the calculated disease intensity (pustules per 40 plants) and X is the lithium concentration in the laminae (mg. Li/100 g. lamina fresh weight). The scatter of points and the two graphs are represented in Fig. 3. The concentration of lithium in the laminae which, if present, should reduce the disease susceptibility to zero was found to be 40·6 mg. Li/100 g. lamina fresh weight (using equation (ii)) which is a little higher than any concentration recorded.

Discussion

Nature of the stimulatory effect. Wortley (1936) emphasized the importance of maintaining optimum conditions for development of disease. The fair test of a fungicide is that it controls the fungus without harming the host plant. The concentrations of lithium used in these experiments were restricted to those which produced growth stimulation coupled with reduction in susceptibility to disease. In this paper 'stimulation' is applied to an

apparently favourable alteration in host-plant metabolism demonstrated most easily by an increase in dry-matter production. It can be caused by small doses of a toxin, by bacterial and fungal attack or by wounding, etc. Stimulation due to one cause, e.g. high temperature, may be antagonistic to that due to another, e.g. injury (Thoday, 1913), suggesting that all types of stimulation are not the expression of any one mechanism in the host plant. This paper is concerned with stimulation produced by small amounts of lithium.

There is abundant evidence of stimulation caused by certain inorganic salts (Voelcker, 1900–12). These results show that there is considerable variation between the effects of different salts of the same metal, particularly regarding the concentrations which produce respectively maximum stimulation and toxicity. Stimulation involves other phenomena as well as dry-matter production; for example, Copeland (1903) found that, with small doses of a toxin, respiration varied in a similar manner to yield. He considered that respiration provided a truer index than growth of the plant's activity. Growth was dependent on respiration for energy and ordinarily they varied together. When they did not, it was the respiration which revealed the plant's true activity. Various workers have found that the respiration of higher plants was stimulated by organic toxins such as chloroform (E. P. Smith, 1924) and by inorganic salts such as potassium chloride and nitrate (Jacobi, 1899). Watterson (1904) found that when *Aspergillus niger* was treated with 0·16 % LiCl (=0·038*N*) the CO_2/fungus dry weight ratio was increased from 0·83 to 1·04. Lithium is not an essential element; it cannot satisfactorily replace potassium in the higher plants (Scharrer & Schropp, 1933), and plants grow satisfactorily in its complete absence. Lundegårdh (1931) tried to explain the stimulatory effect of some 'non-essential' elements from the standpoint of colloid chemistry. He suggested that aluminium, which promotes growth when present in small quantities (Stoklasa, 1922), probably had the same function as calcium, removing the toxic effect of other ions (e.g. copper) by reason of its strong colloidal properties. It is doubtful if this explanation can be applied to lithium, a monovalent ion, whose colloidal properties are considerably weaker than those of aluminium.

It has been suggested that the stimulant increases permeability to oxygen and carbon dioxide. Thoday (1913) showed that in mild cases of stimulation of respiration by chloroform the CO_2 output and the O_2 intake were simultaneously increased, indicating close correlation, a result possibly due to increased protoplasmic permeability. With large doses there was disorganization and the close correlation broke down—diminished CO_2 output was accompanied by a rapid inrush of O_2; the transition between the two was so sharp that intermediate doses might cause disorganization in one part of the leaf but not in another. Leaf proliferation is apparently a stimulative effect and necrosis a toxic sympton, yet tobacco mosaic virus causes a proliferation of the leaves of *Nicotiana tomentosa* and *N. paniculata*, while on another host, *N. rustica*, it causes systemic necrosis (K. Smith, 1933), suggesting that the stimulative and toxic effects are more closely related than they would at first sight appear. This close relation is reminiscent of the classical katalytic theory of stimulation due to Richards (1899), who demonstrated stimulation or 'chemical irritation' of fungi by salts of zinc, nickel, manganese and lithium. Copeland (1903) pointed out that a supraoptimal increase in respiration, caused by rise of temperature or chemical stimulus, could be regarded as the cause of a decrease in growth. Brenchley (1910) found that concentrations of manganese which stimulated the vegetative growth of cereal plants still retarded the ripening of the grain. In the experimental results quoted above it was shown that growth

of celery-plant tops might be stimulated while root growth was retarded; it is apparent from a study of the concentration of lithium in the various parts of the plant that such a coincidence would be expected.

Nature of the toxic effect. Symptoms of lithium toxicity were first shown in the marginal part of the lamina which dried out and bent over, changing in colour to greyish green and later to pale yellow. Subsequently the whole leaf was affected. The toxicity of lithium, applied so that it can be absorbed by the roots, depends not only on its chemical properties but on such factors as the kind of culture (water, sand or soil), the relative abundance of essential nutrients and the ultimate fate of the toxin in the plant. Plants grown in water cultures containing nutrient salts can endure a much greater concentration of toxin than in the absence of the nutrient (Brenchley, 1910). The effect is more marked in soil cultures, since the toxicity of an added constituent is reduced owing to adsorption (Brenchley, 1932, 1938). Hence, in these experiments, toxicity was related to the lithium concentration in the plant rather than to the amount added to the soil. The fate of toxic elements inside the plant depends partly on the chemical and physical properties of the toxin. Lithium accumulates in the laminae of celery plants (see Table 3), and Haas (1929) stated that a toxic agent, such as lithium, which is not readily precipitated within the conducting system of the tree, concentrates in the leaves, after equilibrium is established, as a result of transpiration. Nickel and cobalt accumulate in leaves and seeds (Brenchley, 1910); arsenic and barium occur mainly in the roots (Colin & De Rufz, 1910; Hurd-Karrer, 1939) and aluminium in the roots and trunk of trees, but only to a very small extent in the leaves (Brenchley, 1932). The relative toxicity of various elements also depends on the species of plant: thus, Brenchley (1938) found that the toxicity of copper, cobalt and nickel to barley was in the order Cu > Co > Ni, whereas their toxicity to beans was in the order Co > Ni > Cu.

In these experiments reduction of growth was closely related to a high concentration of lithium in the leaves; since the leaves were not analysed for the essential mineral constituents, it is impossible to say whether the plants, in addition to having excess lithium, were deficient in calcium or potassium. It is well known that lithium and calcium are antagonistic to each other; both lithium and magnesium are toxic to plants which need calcium (Frerking, 1915) and the toxicity of lithium and magnesium to such plants is alleviated by supplying calcium (completely in the case of magnesium toxicity and partly in lithium toxicity; Pirschle, 1932). Frerking (1915) considered that lithium or magnesium replaced calcium, but Haas (1929), who found an excess of calcium in the sap of the leaves of citrus trees on which mottle-leaf disease had been induced by lithium treatment, suggested that lithium made the calcium unavailable to the plant; Frerking assumed that an antagonism between lithium and calcium took place outside the plant, whereas Haas supposed that it occurred inside. The effect of stimulants on protoplasmic permeability (viz. the breakdown of closely related CO_2 output and O_2 intake; see above) may be a factor in lithium toxicity; the effect of lithium on enzymes may also be important, since Gerber (1911) showed that, whereas slight traces of rubidium and caesium chlorides accelerated, lithium chloride in all tested concentrations retarded the action of proteolytic enzymes.

Reduction of susceptibility to leaf spot. The effect of lithium in reducing the amount of leaf spot on celery can be considered from two points of view: toxicity to the fungus and reduction of host-plant susceptibility, and in relation to the habit of the fungus—penetration, situation of the mycelium in the leaf and contact between host and parasite. It is not easy

to see how presence of lithium in the host cells can influence passage of the penetration hyphae through the stomata, since in this type of penetration the fungus does not come into close contact with the host cells. The number of penetrations might be reduced if more of the stomata were closed, but there is no direct evidence that presence of lithium in the plant has any effect on stomatal closure. Lithiated plants had a water content greater than that of the controls, and although this might have been due to reduced transpiration following stomatal closure it seems more likely to have been the result of increased water uptake. Within the leaf the mycelium is situated in intercellular spaces between the cells and food is withdrawn from the host without the aid of haustoria. It is possible that in withdrawing food the fungus receives some lithium which may accumulate until the concentration becomes toxic and retards fungal growth. It would be difficult to estimate the lithium concentration in the intercellular mycelium, and information, which could readily be obtained, regarding the lithium concentration in the fungus grown on a lithiated artificial medium would hardly be relevant, since the growing conditions in the leaf and on agar are so different. Lithium may interfere in some way with the food supply of the fungus, by causing anatomical alterations of the host cell walls or by retarding or by changing the end products of enzymatic action. An analysis of lithiated and control plants for sugars and other organic constituents might throw light on this point.

It is clear from the results of these experiments that lithium must be present locally where the fungus is situated in order to reduce susceptibility; in lithiated plants fewer pustules were found on the marginal areas of the leaves where lithium accumulated. The reduced susceptibility persisted only a short time after a single application, whereas staggered applications caused the effect to persist, as shown by a more constant C/L ratio. Rumbold (1921), who attempted to control *Endothia parasitica* by injecting carbonate and hydroxide of lithium into trees, also found the treatment effective at first, but later, when the lithium was eliminated, the trees became susceptible. Miller & Mitchell (1931) found more efficient absorption of manganese from frequent small doses than from one large dose. Brenchley (1936), commenting on these results, suggested that manganese might be rapidly deactivated in the soil. A prolonged reduction of susceptibility was noticed in the author's experiments following staggered applications of lithium; while it is possible that, in the case of a single large dose, deactivation in the soil occurs in a similar way to that reported by Brenchley, it seems probable that this explanation is not valid for lithium since the latter is not readily precipitated and is absorbed more rapidly from dilute than from concentrated solutions. In addition to a prolonged reduction of susceptibility, staggered applications of lithium had a milder toxic effect than single large applications, the toxic effect of which was severe and the effect upon the susceptibility intense but not so persistent; this suggested that the two effects, toxicity and reduction of susceptibility to disease, are not closely related.

Wheat and mildew

The objects of the experiments with lithium on wheat and powdery mildew (*Erysiphe graminis* DC.) were to confirm the results obtained by Wortley (1936, 1938) that lithium decreased susceptibility of wheat to mildew, and to extend that work by relating disease intensity to concentration of lithium in the wheat plant tissues. Spinks (1913) and Reed (1915) showed that 0·001–0·003 % of lithium in the soil reduced mildew intensity on wheat. Vavilov (1919) also showed that 0·003 % of lithium in the soil reduced the susceptibility

of wheat to mildew but observed no change when lithium was used in sand cultures. Wortley (1936) confirmed Spinks's observations regarding mildew on wheat, but none of these workers analysed the plant tissues for lithium. Voelcker (1900–12) showed that at concentrations of 0·0025–0·001 % of lithium in the soil the yield of grain and straw of wheat and barley was considerably increased. Lithium is not an essential element, so this improvement in growth could be regarded as 'stimulation'. Voelcker showed that lithium in supraoptimal amounts was very toxic, particularly to the root system of young seedlings. Hahn (1916) found that 0·025 % of lithium nitrate in a nutrient solution retarded the growth of wheat after 3 months. Wortley (1938) suggested that toxicity of lithium might be due to interference with the use of calcium in the leaf, and stimulation, to the influence of lithium on permeability of cell membranes to substances concerned in respiration.

Experimental results

Data concerning relative disease intensity, yield and lithium content, from five experiments involving about 500 plants, are summarized in Table 7. The relative disease intensity decreased fairly regularly as the lithium dose was increased (the smallest dose was an

TABLE 7. *Effect of lithium chloride and nitrate upon relative disease intensity of mildew on wheat yield and lithium content of the wheat seedlings*

Milli-equivalents of added Li/l. soil	Salt used	Rel. disease intensity: anion		Rel. dry wt.: weeks of treatment		Conc. of Li in tops: mg./100 g. material		Li content of tops: μg. per plant	Corrected % uptake of Li by tops
		Cl	NO_3	3	10	Fresh	Dry		
0·00	None	100	100	100	100	2·81	23·6	5·6	—
0·25	NO_3	—	71	135	—	—	—	—	—
0·75	Cl	88	—	145	154	3·72	32·4	10·7	1·7
1·26	NO_3	—	86	132	—	—	—	—	—
1·84	Cl	73	—	—	142	—	—	—	—
3·67	Cl	66	—	126	85	10·0	94·0	27·0	1·4
6·30	NO_3	—	46	129	—	—	—	—	—
7·33	Cl	34	—	—	66	—	—	—	—
18·35	Cl	15	—	107	43	18·4	157·6	38·2	0·6
36·7	Cl	—	—	—	50	—	—	—	—

exception), regardless of the anion of the salt used. The coefficient of correlation between lithium supply and disease intensity was $-0{\cdot}908$ ($n=6$, $P<0{\cdot}01$). The effectiveness, particularly of the smaller doses, was considerably increased when the time between lithium application and inoculation was lengthened to 10 days. Small doses of both chloride and nitrate steadily increased the yield of the seedlings, whereas large doses stimulated at first but later became toxic and reduced the yield. It is probable that both stimulative and toxic effects following a single lithium application are transitory and only become permanent if the appropriate dose of lithium is applied repeatedly. Small quantities of lithium were present in the untreated plants. Both lithium concentration in the tops and total content of lithium were increased when lithium was applied, but not proportionally, since the corrected uptake (see Table 8) showed a steady decrease with larger doses. (The uptake figures were corrected for the small amount of lithium present in the controls.) The coefficient of correlation between lithium concentration in the fresh material and relative disease intensity was $-0{\cdot}96$ ($n=2$, $P<0{\cdot}05$).

Discussion

The relation between lithium concentration in the fresh material and relative disease intensity was approximately linear. From the regression equation it was deduced that at a concentration of about 30 mg. Li/100 g. fresh material the relative disease intensity would approximate to zero. This is higher than the concentration found 20 days after application of the largest dose, but evidence has been obtained from other experiments (Kent, 1940) that fresh-weight concentrations considerably surpassing this figure were produced by the highest concentrations here used (18·35 mg.-equiv./l. soil) after a period of 7 weeks. The growth after that time was, however, seriously reduced. These experiments have shown that a low relative disease intensity of mildew on wheat seedlings is associated with a high concentration of lithium in the leaves. Information concerning the localization of lithium in the leaf is still lacking, principally owing to technical difficulties. It is therefore impossible at present to say whether the fungus, when invading the leaf, comes directly into contact with a high lithium concentration. *Erysiphe* penetrates by cuticular puncture and enzymatic action upon the subcuticular wall (Corner, 1935), and penetration of lithiated plants is arrested when the subcuticular wall is partly dissolved (Wortley, 1938). Wortley suggested from cytological evidence that lithium reduced susceptibility to mildew either by altering the subcuticular wall of the host (immunizing it to fungal enzymes) or by acting upon the enzymes directly.

Application of potassium, an alkali element analogous to lithium, has long been known to reduce susceptibility to disease. Spinks (1913) found that wheat manured with potash had less mildew. Stuck (1926) confirmed this and showed that the sclerenchyma and parenchyma cells of plants manured with potash had thicker walls, but he found no correlation between wall thickness and susceptibility to mildew. Arland (1931) also showed that barley, manured with potash, was less susceptible to mildew and that the epidermal walls were thicker. Schaffnit & Volk (1930) found that potash manuring produced small-celled tissues and retarded transport of assimilated materials. Chona (1932), experimenting on the decomposition of potato disks by fungal enzymes, found that by soaking disks in solutions of potassium or magnesium salts the time taken for decomposition was increased, and he suggested that these salts had a hardening effect. Němec (1936) analysed potato varieties resistant and susceptible to wart, and also normal and warted potatoes; he found that in resistant and in normal potatoes the potassium and magnesium contents were higher than in those which were susceptible or warted. Apparently potassium has specific functions in the plant that cannot be performed by lithium, sodium or rubidium. Loew (1903), who enumerated certain of these functions, regarded its condensing functions as most important, and Weevers (1911) considered that potassium regulated turgidity. It is possible that the absorption of a large amount of lithium is accompanied by reduced absorption of potassium according to the mass-action hypothesis (Hurd-Karrer, 1939), and in this case the reduction of susceptibility induced by lithium might be due either to the presence of lithium in the plant or to deficiency of potassium or both. Data concerning localization of lithium in the epidermis, possible chemical or mechanical alteration of the wall and interaction of lithium with fungal enzymes are required before it can be established whether the mechanism of susceptibility reduction is direct or indirect.

Wheat and brown rust

Wortley (1936) endeavoured to reduce susceptibility of wheat to brown rust (*Puccinia triticina* Erikss.) by application of lithium salts. He found that lithium had only a slight effect on the disease and at concentrations (37 mg.-equiv./l. soil) which were toxic to the wheat. *Erysiphe graminis* parasitizes the plant by puncturing the cuticle and dissolving the cellulose epidermal wall (Corner, 1935), whereas both *Septoria Apii* and *Puccinia triticina* enter through the stomata (Klebahn, 1910; Caldwell & Stone, 1936). Inside the leaf, *Septoria* mycelium ramifies in the intercellular spaces, making no very intimate connexion with the host cells (Klebahn), whereas *Puccinia* obtains food through haustoria in the mesophyll cells. If lithium treatment induced resistance to *Septoria*, there seemed no a priori reason why resistance to *Puccinia* should not be similarly imparted. Experiments were therefore performed to confirm or disprove Wortley's results, that lithium could not reduce susceptibility to brown rust of wheat without seriously harming the host plants.

Survey of literature

References in the literature to experiments with lithium on rust are scanty. Raines (1922) quotes Voelcker's work at the Woburn Pot-culture Station and asserts that he showed susceptibility to rust was depressed by lithium. Voelcker (1900–12), however, performed no experiments with fungal diseases and recorded the effect of lithium only on the yield of plants. Brown (1936) states that lithium salts markedly increased rust resistance and quotes Spinks as his authority. Spinks (1913), however, who examined the Woburn pot cultures with a view to finding the effect of lithium on rust and mildew, found that rust was practically absent on those plants, 'the amount present', he stated, 'being so small that no idea of the susceptibility of the various cultures could be gained'. The only experiments with lithium on wheat and brown rust known to the author are those of Wortley (1936).

Wortley (1938) was unable to reduce susceptibility to rust by applying potassium salts, whereas other workers have reported a reduction of rust incidence following potash manuring (e.g. Spinks, 1913; Gassner & Hasserbrauk, 1931). Raines (1922) considered that the virulence of the rust parasite was directly related to the vigour of the host (as measured by dry-weight yield), and Gassner & Goeze (1932) related this to the effect of potash manuring by showing that application of potassium reduced the amount of material assimilated and hence raised resistance to rust. Nightingale *et al.* (1930) also showed that plants deficient in potassium often accumulated much carbohydrate owing to a reduced rate of nitrate assimilation. The soluble carbohydrate content would appear to be an important factor in the establishment of an obligate parasite in the host, and Singh & Prasad (1936) showed that 0·005 *M* lithium chloride in water culture increased the total carbohydrate content of wheat and shifted the balance towards the insoluble starch.

Experimental results

The results of experiments involving about 140 plants are presented in Table 8. There were three treatments—control and lithium chloride at 7·3 and 18·3 mg.-equiv./l. soil. The doses required to produce these concentrations were 10 c.c. of 1 and 2·5 % lithium chloride respectively per 4 in. pot of soil. The reduction of 28 % in relative disease intensity with the treatment of 18·3 mg.-equiv./l. was significant ($t = 5{\cdot}7$, $n = 6$, $P < 0{\cdot}01$); the reduction

of 10 % with the weaker dose was not. However, the concentration required to produce a significant reduction of disease reduced the dry-weight yield to less than half that of the controls. No significant reduction in disease was induced by smaller doses than these, and it was not found possible to produce any significant reduction of disease without seriously reducing the yield.

TABLE 8. *Effect of lithium chloride on density of brown rust on wheat seedlings*

Mg.-equiv. Li applied/l. soil	No. of leaves on which disease was estimated	Total no. of pustules counted	Total length of diseased leaf measured (cm.)	Av. no. of pustules per cm. length of leaf		Rel. disease intensity	Rel. dry wt. (after 10 weeks)
0·0	26	3858	91·9	42·0	41·9	100	100
0·0	26	3572	85·5	41·8			
7·3	32	4754	126·9	37·5		90	66
18·3	28	2647	78·8	33·1	30·3	72	43
18·3	31	2950	105·9	27·8			

Discussion

The results of these experiments with brown rust of wheat stand in contradistinction to those with leaf spot of celery; the intensity of the latter could be substantially reduced (see Table 2) by applications of lithium which were beneficial to the celery plants. The difference between the two fungi *Puccinia triticina* and *Septoria Apii* and their hosts in their reaction to lithium treatment may be explained by the fact that *Puccinia* is an obligate parasite, closely restricted in host range, whereas *Septoria* is a facultative parasite which can also be cultivated on artificial media. Raines (1922) considered that an obligate parasite such as rust, which lives 'symbiotically' with its host, attacks best when the host is growing most vigorously, whereas a facultative parasite such as *Septoria Apii*—which was considered by Cochran (1932) to secrete a toxin, killing the host on which it existed saprophytically—would be favoured when the host was weak. It is suggested that in the case of rust lithium is toxic to the fungus in the leaf and only so when a high lithium concentration has been attained, such as is also toxic to the host; that in the case of leaf spot lithium increases resistance of the host, possibly by inhibiting toxic substances or enzymes secreted by the fungus.

TOMATO AND CROWN GALL

Survey of literature

Sempio (1934) studied the effect of the nitrates of nineteen metals on the growth of *Ricinus* seedlings, the development of *Bacterium tumefaciens* tumours on *Ricinus* and the growth of the bacterium in culture. He found that the effects on the seedlings, the tumour and growth in culture showed no strict relationship to each other although salts which depressed growth of the tumour were also toxic to the bacterium in culture. On the other hand, Gosset *et al.* (1934) showed that *Bacterium tumefaciens* galls on *Pelargonium zoneale* were killed after the cut stems had been immersed in 0·018 % germanium oxide for 1 week, whereas the same concentration did not prevent growth of the bacterium in culture. Sempio used only a few concentrations of each salt; those which he reported as inhibiting tumour development might well have been innocuous or even stimulatory at lower concentrations, as his results with mercury would suggest. He suggested from the lack of co-ordination between the effects on tumour development and on the bacterium in culture that the former was due to an effect on the host-parasite complex rather than on the bacterium in the host.

Cook & Halferdahl (1937), experimenting with lithium phosphate as a weed-killer, reported that 50 p.p.m. lithium (as phosphate) mixed with the soil reduced the yield of tomato plants to 37 %. Ravenna & Maugini (1912) observed that the sensitivity to lithium was greater for tomatoes than for other plants with which they were experimenting. Maasen (1904), examining the effect of alkali chlorides on the growth of bacteria on nutrient agar, found that lithium caused giant forms and abnormal shapes to develop, and that a mucilage covering was produced which acted as a 'preservative'.

Experimental results

The influence of lithium on gall size and fresh-weight yield of tomato plants is shown by the results of three experiments (Table 9). Increasing concentrations of lithium (chloride or nitrate) caused a decrease in gall size; in the nitrate experiment the effect of lithium on

TABLE 9. *Gall size and weight, and fresh-weight yield of tomato plants treated with lithium*

Mg.-equiv. Li applied/l. of soil (*a*)	Salt used (*b*)	Visual estimation of gall size (*c*)	Net gall diam.(mm.) (*d*)	Rel. gall diam. (*e*)	Mean fresh wt. of gall (g.) (*f*)	Rel. gall wt. (*g*)	Rel. fresh wt. yield	
							Tops (*h*)	Roots (*j*)
			(i) 10 weeks after treatment					
0·00	Cl	100	8·71	100	0·359	100	100	100
0·73	,,	94	8·75	101	0·352	98	106	96
1·83	,,	86	8·85	102	0·336	94	71	108
			(ii) 13 weeks after treatment					
0·00	NO_3	—	8·8	100	0·57	100	100	—
0·025	,,	—	9·4	108	0·67	119	99	—
0·13	,,	—	8·5	96	0·46	81	95	—
0·25	,,	—	9·0	102	0·47	83	97	—
1·3	,,	—	7·3	82	0·35	61	102	—
2·5	,,	—	5·5	63	0·22	38	101	—
			(iii) 17 weeks after treatment					
0·00	Cl	100	8·43	100	0·282	100	100	100
0·55	,,	96	7·85	91	0·233	82	118	135
1·10	,,	86	8·02	95	0·252	89	147	106
2·20	,,	75	6·84	81	0·239	85	107	114

gall diameter and weight was found by analysis of variance to be significant ($P<0·01$). There were significant differences—20·4 % of the controls for diameter (col. *e*) and 54 % for weight (col. *g*)—between the control and the 2·5 mg.-equiv./l. sets. The increase caused by 0·025 mg.-equiv./l. treatment was not significant. The three experiments were of different durations and their results are therefore not comparable. The yield of tomato plant tops (col. *h*) was practically unaltered by any of the lithium nitrate treatments. The smaller lithium chloride treatments had a slight stimulatory effect, whereas the larger doses were toxic at first; subsequently the toxicity decreased and the treatment became stimulatory. Root yield was not recorded in the nitrate experiment, but all the chloride treatments appeared to be beneficial to root growth. There appeared to be little relation between yield of the tops and size of the galls.

Gall *weight* estimations cannot be used to follow the influence of lithium on the development of the gall, whereas this can be done by making successive gall *diameter* measurements. The coefficient of correlation between gall weight and gall diameter (cols. *d* and *f*) was

+0·896 ($n=38$, $P<0·01$). Assuming that weight is the best criterion of gall size, this suggests that results obtained by using gall diameter measurements would present a close similarity to those obtained from gall weight measurements. Lithium nitrate was more effective in reducing weight of the galls than was lithium chloride of approximately equivalent concentration. The results are, however, not directly comparable, since the nitrate experiments were done in summer whereas the chloride experiments were carried out in winter, when the galls grew more slowly. Galls on the untreated plants contained a small amount of lithium (see Table 10, cols. *c*, *d*), and its concentration was considerably increased by lithium treatment. In order to correct for lithium in the controls, the 'concentration increase as compared with the controls' was obtained by subtracting the figure for the ash-weight concentration in the untreated plants from that in the treated (col. *f*). The coefficient of correlation between 'concentration increase' and the concentration of added lithium (col. *a*) was +0·997 ($n=3$, $P<0·01$), showing a close relationship. The mean gall weight (col. *b*) decreased steadily as the lithium concentration in the galls increased (col. *f*). The correlation between gall weight and lithium concentration in the fresh gall (col. *c*) was

TABLE 10. *Gall weight, lithium concentration in galls and dead leaves, and loss of leaves from tomato plants treated with lithium nitrate*

Mg.-equiv. Li nitrate applied/l. of soil (*a*)	Mean gall wt. (mg. fresh wt.) (*b*)	Li conc. (mg. per 100 g.) in Galls: Fresh wt. basis (*c*)	Galls: Ash wt. basis (*d*)	Dead leaves: Ash wt. basis (*e*)	Increase in Li conc. (compared with controls): mg./100 g. ash: Galls (*f*)	Leaves (*g*)	Leaves lost per plant in 22 days: g. dry wt. (*h*)	Ratio of ash conc. of Li: Leaves/Galls (*j*)
0·00	537	0·26	12·5	52·3	—	—	0·30	4·2
0·025	675	0·26	12·5	57·5	0·0	5·2	0·46	4·6
0·13	652	0·34	15	71·2	2·5	18·9	0·35	4·7
0·25	588	0·41	20	131·3	7·5	79·0	0·36	6·5
1·3	385	0·76	35	383·5	22·5	331·2	0·52	10·9
2·5	217	1·49	60	650·0	47·5	597·7	0·63	10·8

$r=-0·956$ ($n=4$, $P<0·01$). The regression of gall fresh weight (mg.) on lithium concentration in the fresh gall (mg./100 g.) was found to be $Y'=710·31-344·5X$, where Y' is the calculated gall fresh weight and X is the lithium concentration found. If this relationship is assumed to persist for higher values of X, it can be deduced that the size of the galls will approach zero as the lithium concentration in the gall approaches 2·06 mg./100 g. fresh material.

Plants with the higher lithium treatments shed their leaves prematurely, but these plants subsequently made better growth, suggesting that the lithium was being excreted in the dead leaves; accordingly, the dead leaves were collected and analysed. The lithium concentration in these leaves (Table 10, col. *e*) increased steadily with increasing lithium application; the correlation between the concentration increase (col. *g*) and the concentration applied (col. *a*) was $r=+0·998$ ($n=3$, $P<0·01$). Lithium treatment accelerated leaf fall (see Table 10, col. *h*): this, together with increased lithium concentration in the leaves, enabled the plants to excrete a considerable quantity of lithium. The fluctuation in the ratio between the lithium concentrations in the ash of leaves and galls (col. *j*) shows that as the lithium application was increased, relatively more lithium was stored in the leaves than in the stem (assuming galls to be a fair sample of stem). The effect of lithium on the growth

of *Bacterium tumefaciens* in artificial culture was investigated by adding lithium to the agar culture medium. To each plate of 25 c.c. of medium (1·5 % agar, 1·5 % malt extract) 0·1 c.c. of lithium sulphate solution was added to give concentrations of 4, 0·4, and 0·04 mg. Li/l. of medium. The diameter of the colonies (produced by inoculation with a needle held vertically) was measured after 5 days; measurement was made in two directions at right angles and the mean diameter recorded. The mean diameters of nine colonies per treatment were respectively 8·4, 7·6, 8·8 and 8·5 mm. for the treatments 4·0, 0·4, and 0·04 mg. Li/l. and control, suggesting that these concentrations of lithium had practically no effect on the growth of the bacterium in culture.

Discussion

The mechanism of the lithium effect on crown gall disease would seem to be different from that of the fungal diseases studied. In fungal infection the critical events are the entry of the fungal germ tube and the establishment of haustoria within the host cells (mildew and rust), or of the mycelium in the intercellular spaces of the host tissue (leaf spot). In the case of crown gall, however, the pathogen enters the host through a wound produced naturally or by inoculation; the bacteria do not enter the wounded cells but exert their influence through the intercellular liquid (Riker, 1923). The critical event in the success of gall disease apparently is the production of growth substance responsible for initiating the formation of galls (Locke *et al.* 1938). Locke *et al.* (1939) grew cultures of gallforming and non-gallforming bacteria, in all of which growth substances were produced in approximately equal amounts. Hence they suggested that the amount of growth substance produced in culture has no direct major relation to the pathogenicity of crown gall bacteria. Leonian (1937), reviewing Boysen-Jensen's work, pointed out that galls may equally well be the result of excessive production of growth substances by the host tissues under the influence of the bacteria as of growth substances furnished by the pathogen to the host plant.

Lithium treatment increased the concentration of lithium in the gall, and there was a high negative correlation between that and the gall size. Lithium may directly influence the bacteria in the host, or it may inhibit manufacture of the growth substance responsible for gall formation. The highest concentration of lithium found in the galls (1·49 mg./100 g. fresh wt.; see Table 10, col. *c*) surpassed the highest concentration of lithium used in the culture-medium experiments (4·0 mg./l.); the former considerably reduced gall size, whereas the latter had no apparent effect on growth of the bacteria. Since higher lithium concentrations in culture medium were not used, it cannot conclusively be shown from these experiments whether the lithium acts upon the bacteria directly or indirectly. Lithium in culture medium is distributed evenly, but lithium in the gall may be concentrated in certain parts, and to such an extent that it is toxic to the bacteria. This possibility cannot be finally dismissed until more information is available concerning the localization of lithium in the gall and the nature of, and the influence of lithium on, the growth substance which presumably initiates gall development.

Summary

1. A study was made of the effect of lithium chloride and nitrate on the susceptibility of celery, wheat and tomato plants to certain parasitic diseases, on growth of the host plants and on the concentration and distribution of lithium in their tissues.

2. Lithium chloride and nitrate at concentrations between 1 and 4 mg.-equiv. Li/l. soil reduced the amount of leaf spot disease (*Septoria Apii*) on celery and at the same time increased the weight of the host plants; larger concentrations had a more pronounced effect on the disease but were toxic to the host plants. The lithium content of the plants was increased by lithium application to the soil; there was a high inverse correlation between lithium concentration in the celery laminae and amount of disease present.

3. Application of lithium chloride and nitrate to wheat seedlings reduced their susceptibility to powdery mildew (*Erysiphe graminis*), in small doses stimulated, and in large doses retarded the growth of the seedlings, and at all tested concentrations increased the lithium content of the plant tops. Significant inverse correlations were found between relative mildew intensity and both lithium supply in the soil and lithium concentration in the fresh material.

4. The susceptibility of young wheat plants to brown rust (*Puccinia triticina*) was significantly reduced by adding lithium chloride to the soil at the rate of 18 mg.-equiv./l. soil; this concentration was distinctly toxic to the wheat plants, reducing their dry-weight yield to less than half that of the control plants. Smaller concentrations had no significant effect on the disease.

5. The diameter and weight of tomato crown galls (*Bacterium tumefaciens*) were significantly reduced by lithium nitrate applied at the rate of 2·5 mg.-equiv./l. soil. The fresh weight of the plants treated with lithium chloride showed an increase compared with untreated plants when measured 17 weeks after lithium application. The effect of lithium nitrate on host plant yield, at the concentrations tested, was negligible. There was a close relationship between the lithium concentration in the galls and that applied, and a high inverse correlation between gall weight and concentration of lithium in the gall. Excretion of lithium from the treated plants was increased by the high concentration in the leaves and by premature leaf fall.

6. The nature of the reduction of susceptibility, the stimulatory and the toxic effects is discussed.

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FUNGI WHICH CAUSE PRE-EMERGENCE INJURY TO GARDEN PEAS

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(With Plate 10 and 2 Text-figures)

In 1937 Hull described the occurrence of pre-emergence damping-off in sowings of peas made at the Biological Field Station, Slough, over two seasons (1933–5), together with an account of a chemical control measure and a preliminary statement regarding the pathogenic fungi involved. The work was continued by the writer over a further two years (1936–8), and the following account deals with the mycological aspects of the problem.

According to Hull (1937) the organisms responsible for failure of emergence were a species of *Fusarium* and a phycomycetous fungus, which was not further identified, and of these the latter was the more virulent. Numerous workers in America (e.g. Haenseler, 1925; Jones & Linford, 1925; Horsfall *et al.* 1934; Anderson *et al.* 1937) and some in Europe (e.g. Buisman, 1927) have stressed the significance of *Pythium* spp. in this connexion. The status of *Fusarium* spp. is uncertain, but there is general agreement (Jones, 1923; Haenseler, 1925; Snyder, 1933; Crosier, 1936) that species which cause wilting or foot-rotting of the growing plants have little effect in depressing the emergence of seedlings. On the other hand, damping-off of peas in England has been ascribed to *Fusarium* spp. by Brett *et al.* (1937) and by Padwick (1938). *Ascochyta Pisi* and related fungi, whatever damage they may cause subsequently, do not seriously reduce the emergence of peas (Crosier, 1936; Hull, 1937; Padwick, 1938), and the same is true of the root-rot organism *Aphanomyces euteiches* (Jones & Drechsler, 1925). Some American workers (Richards, 1923; Horsfall *et al.* 1934; Crosier, 1936) state that an important cause of damping-off is *Rhizoctonia Solani*. Finally, the physiological disease known as 'Marsh Spot' has been shown by Lacey (1934) and Pethybridge (1934) to reduce germination, but not to a serious extent unless the disease is present in a severe form.

There is general agreement that emergence of peas tends to be poorest in cold wet soils. Whereas the effect of soil temperature has been inadequately studied, more especially as regards the action of the various pathogens, it has become clear that soil moisture is the predominating factor (Haenseler, 1925; Hull, 1937; Horsfall, 1938), viz. the higher the soil moisture the more severe the disease. In general, round-seeded (starchy) varieties are more resistant to damping-off than those with wrinkled (sugary) seeds.

The nature of pre-emergence injury

The lay-out of field experiments was essentially the same as that described by Hull (1937). Certain rows were set aside for mycological observation, and of these one-half were dug up when the radicles had just grown out, i.e. 1–3 weeks after sowing, according to the speed of germination. The remainder were examined after 2–6 weeks, when the first plants were beginning to show above ground. A healthy seed was readily found, but the soil adhered to a rotted one so that it was easily overlooked. By the time of the second sampling, some seeds had completely disappeared presumably because they had begun to decay at an early stage.

In all, some 4500 seeds were sown for inspection in this way. A typical set of results is given in Table 1, the figures being percentages of the number of seeds sown. The data of average emergence were obtained from rows in the experimental block which were not disturbed. The results of both samplings in 1937 are included, but of the first sampling only in 1938—when the disease was much more pronounced. In the latter year a large proportion of the seed, especially of the wrinkle-seeded variety Gradus, had disappeared by the time of the second sampling. In classifying the embryos attention was chiefly paid to the state of the seedling axis. Lesions on cotyledons only were so common, especially when germination was slow, that they were ignored except when they were so pronounced as to lead to a stunting of the axis.

Comparison of columns 2 and 4 of Table 1 shows that there is substantial agreement between the average emergence and the number of healthy seedlings in the samples taken. As the figures for 1938 refer to the first sampling only, it is evident that the Gradus seed was already so severely attacked as to account for the proportion of seed which failed to emerge. This conclusion is supported by the result of the second sampling which showed that most of the material was either healthy or very much decayed. The discrepancy in 1938 between the number of healthy Pilot (round-seeded) embryos and the average emergence was probably due to the survival of some of the seeds damaged by animals.

TABLE 1. *Condition of pea seeds dug up in the course of germination*

Variety	Av. emergence	Recovered	Healthy	Stunted	Rotted ungerminated	Plumule rotted	Radicle rotted	Plumule and radicle rotted	Animal injury
Pilot, 1937	77	94	76	3	5	3	2	2	3
Gradus, 1937	81	96	80	1	2	6	1	0	6
Pilot, 1938	75	92	67	0	3	5	0	1	16
Gradus, 1938	36	80	41	3	20	4	4	5	3

The type of injury which caused most damage to the Gradus seed in 1938 (col. 6, Table 1) and which also predominated in the Pilot seed of 1937 was a very early rotting of the axis and often also of the cotyledons, occurring when the embryo had produced very slight or no growth. Furthermore, seeds which could not be recovered from the soil would undoubtedly be of this type. It is suggested, therefore, that the period of maximum susceptibility is in the early stages of germination, even before the embryo has broken through the seed-coat. This view is supported by the observations of Jones (1931) and Horsfall (1938), that watering of the seed-bed shortly after sowing markedly reduces emergence, whereas the effect is much reduced if the water is withheld for a few days.

The experimental seed was either untreated or dusted with an organic mercurial preparation. Substantially the same result was obtained in either case, the frequency of seedling rotting being less according to the degree of benefit obtained by the treatment. Very early rotting of the seed predominated even with the most successful treatment. This indicates that the seed decayed when largely or wholly enclosed within the dust-coated testa.

FUNGI PRESENT IN DISEASED MATERIAL

The following methods were used throughout both seasons. After the embryos had been well washed in running water the seedling axes were separated from the cotyledons and vigorously shaken in a test-tube in twelve changes of sterile water. They were then placed on slopes of plain agar and incubated at 20° C. The cotyledons, after the removal of any excessively decayed portions, were dipped in 95 % alcohol, flamed, placed on 0·5 % tartaric acid agar and incubated at 20° C. By these methods a fungus was obtained from 80–100 % of the axes, and from most of the cotyledons when they also

were diseased. The fungi were subcultured on plain agar and an inoculum from the back of the plate transferred to a tube of potato or cornmeal agar. This procedure, which is recommended by Brown (1924), prevented losses from bacterial contamination. These isolations were carried out systematically over two seasons and on material from two soil types—a light sandy loam and a low-lying clay. No marked change occurred in the fungi present as the season advanced or in relation to soil type; the results are therefore summarized in Table 2.

The seedling axes yielded large numbers of cultures of *Pythium* and practically nothing else. *Pythium* spp. also preponderated in the isolations from cotyledons in 1937, but in 1938 were in large measure replaced by a species of *Mucor*. This fungus was absent in the preceding year. Further work showed that it was not seed-borne and that it was not pathogenic. It was virtually confined to the cotyledons. *Ascochyta Pisi* showed a restricted distribution in being practically confined to the Pilot seed in 1937 and the Gradus in 1938. The *Pleospora* sp. occurred only in the former batch. This is explained by the fact that these fungi were seed-borne (see Table 3). *Ascochyta Pisi* and *Pleospora* sp. were the only fungi other than *Pythium* to be detected in diseased axes to any extent. Isolates of *Fusarium* were infrequent, the only substantial occurrence being in the cotyledons of Gradus in 1938.

TABLE 2. *Fungi isolated from peas sown in the field*

	From seedling axis				From cotyledons			
	1937		1938		1937		1938	
Species	Pilot	Gradus	Pilot	Gradus	Pilot	Gradus	Pilot	Gradus
Pythium spp.	61	58	37	79	58	20	6	28
Mucor sp.	0	0	1	1	0	0	16	55
Ascochyta Pisi	14	0	0	2	48	0	1	3
Pleospora sp.	8	0	0	0	24	0	0	0
Fusarium spp.	2	0	2	3	4	2	9	26
Penicillium spp.	0	0	0	0	10	4	9	11
Botrytis sp.	0	0	0	0	8	0	0	0
Rhizoctonia Solani	0	0	0	0	0	1	0	0
Miscellaneous	4	0	0	0	11	7	12	13

Penicillium spp. when present were restricted to the cotyledons. The latter were not studied further in this work, but Padwick (1938) found that the species of this genus tested by him did not reduce emergence. In view of the importance attached to *Rhizoctonia Solani* as a cause of seedling injury to peas in America, its virtual absence from this list is noteworthy.

It should be noted that the material from which *Pythium* was so abundantly isolated was thoroughly washed with sterile water, but not treated with an antiseptic. In view of the observation made by Jones & Linford (1925) and by Buisman (1927) that species of *Pythium* could be isolated with almost equal frequency from healthy as from diseased pea roots if they were not surface sterilized, it was thought desirable to try the effect of surface sterilization of seedling axes with antiseptics. The great sensitivity of *Pythium* mycelium to these is noted by both the authors cited. The treatment considered necessary to kill superficial hyphae is liable to destroy the internal *Pythium* hyphae as well.

If the seedling axes were treated with 0·1 % mercuric chloride and subsequently washed in three changes of sterile water, *Pythium* alone was again obtained; but if the immersion was longer than 45 sec., the number of isolates was reduced. The occasional ones that were obtained even with 3 min. immersion were *Pythium*.

Padwick (1938), who isolated from cotyledons only, surface sterilized these by immersion for 2–3 min. in 0·1 % silver nitrate, followed by a rinse in a saturated salt solution, and

plated the material on potato-dextrose agar. This method was compared with that of the writer, the material being Gradus seedlings with axial lesions. Whereas the majority yielded cultures of *Pythium* after thorough rinsing with sterile water, the silver nitrate method gave no *Pythium* isolates and a high proportion of the seedlings so treated were aseptic.

In order to determine which fungi were carried on the seed samples used, seeds of each batch were washed in twelve changes of sterile water, and others were treated for 3 min. with 0·1 % mercuric chloride followed by three changes of sterile water. The skins, cotyledons and axes were placed separately on plain agar. Only occasional seeds in the samples showed visible lesions. Samples of fifty seeds were also examined separately for Marsh Spot. The results (Table 3) show that the Pilot seed in 1937 was heavily infected with *Ascochyta Pisi*. This and *Pleospora* were obtained several times from the cotyledons and axis. The Marsh Spot lesions were small. The Gradus seed in this year, however, carried little else but *Penicillium*, and this came almost wholly from the skins. In 1938 the Pilot seed was clean, and the amount of *Ascochyta* in the Gradus seed was small.

TABLE 3. *Number of seeds infected in a sample of forty*

	1937		1938	
Species	Pilot	Gradus	Pilot	Gradus
Ascochyta Pisi	18	0	0	4
Pleospora sp.	6	0	0	0
Penicillium spp.	4	11	0	2
Aspergillus sp.	0	0	0	7
Miscellaneous	3	2	0	1
Marsh Spot (50 seeds)	4	0	0	1

The species of *Aspergillus* present in Gradus seed in 1938 was the only seed-borne fungus which did not occur among the isolations made from diseased material from the field (Table 2). It would thus appear to be the least important. The fact that, in the list of isolations from the field, *Ascochyta* was virtually confined to seedlings arising from infected seed stocks, shows that it was not present, or at least was not active, in the soil.

The isolates believed to be species of *Pythium* were plated on cornmeal agar and classified on the nature of the fruiting present into eight groups. All these, but no new ones, were recognized again in the following year. They are listed below together with the criteria by which they were known, and the number of isolates referred to each is shown. The taxonomic work to which reference has principally been made is that of Matthews (1931).

Group A. Abundant sexual organs which agreed with those of *P. ultimum*. No. of isolates: 1937, 37; 1938, 7.

Group B. Sexual organs and conidia agreeing well with *P. de Baryanum*. No. of isolates: 1937, 25; 1938, 32.

Group C. Oogonia few and extremely large, approx. 45μ diam. No. of isolates: 1937, 2; 1938, 0.

Group D. Conidia alone present, spherical, more or less abundant, especially superficially, tending to become highly granular and to develop a thickened wall. Av. diam. about 20μ. No. of isolates: 1937, 82; 1938, 52.

Group E. Conidia alone present, spherical, few and comparatively large, often exceeding 30μ diam. No. of isolates: 1937, 14; 1938, 32.

Group F. Conidia alone present, spherical, intermediate in size and number between groups D and E. No. of isolates: 1937, 13; 1938, 20.

Group G. Sterile Phycomycetes closely resembling members of the preceding classes in general cultural characters. No. of isolates: 1937, 2; 1938, 7.

Unclassified: 1937, 22; 1938, 0.

Representative cultures were inoculated on sterilized carrot and grown in large vessels of water in the hope of inducing the development and discharge of sporangia, so that the genus might be identified by its formal criterion of producing its zoospores in a vesicle. No indication of zoospore formation was however obtained, whereas the asexual reproductive bodies which appeared here, as on agar, had often been seen to produce germ tubes on the latter medium. It was assumed therefore that the fungi were conidia-producing members of the genus *Pythium*, a conclusion supported by the fact that it was possible to refer those cultures which produced the sexual stage within that class. A possible exception is the unimportant group C. In both years the most numerous isolates were those of group D. In 1937 there was also a large number of group A, but in the following year there were very few of these, their place as the second most abundant form being taken by group E.

The cultures selected for further experiments were purified by hyphal-tipping. Bacteria were difficult to eliminate from fungi of this genus but these cultures appeared clean even on neutral bouillon agar.

Pathogenicity of isolates under controlled conditions

An endeavour was made to test the pathogenicity of the more frequently occurring isolates under conditions approaching those which obtained in the field. The following details of technique were observed in all the experiments. The soil-borne fungi concerned were grown at 20° C. for 10–17 days on a sterilized mixture of 95 parts by weight of soil and 5 of cornmeal. This inoculum was stirred into the whole bulk of the soil used for potting at the rate of 4–5 %, and controls received a similar amount of uninoculatd medium. Containers were sterilized by heat or with formalin. Soil was sterilized, inoculated and potted at a water content of approximately 40 % of saturation, the time allowed in the autoclave being 1–3 hr. at 20 lb. pressure.

In a preliminary experiment with representatives of five of the groups of *Pythium* listed above (groups C and G being omitted as relatively unimportant), all caused severe pre-emergence damping-off. In this, and similar experiments, the seed was surface-sterilized with mercuric chloride. The soil was adjusted to approximately 80 % saturation. As the various *Pythium* isolates did not differ widely in the effects produced, the results were combined and were as follows:

	% emergence	
	Pilot	Gradus
Controls	73·5	56·8
Inoculated	3·6	3·9

The imperfect emergence of the controls, especially with Gradus, was presumably due to the excessive wetness of the soil (vide Text-figs. 1 and 2).

The effect of soil moisture and temperature was studied with one isolate from each of the groups A and D, with results as shown in Text-figs. 1 and 2, and Pl. 10.

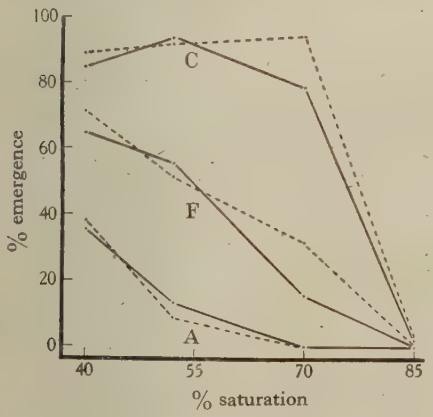
The following points are clearly indicated: the difference in soil temperature did not materially affect the pathogenicity of either of the fungi or the germination of the controls; emergence with both fungi bore a strongly marked inverse relationship to soil moisture, whereas controls did not show this; the isolate of *Pythium* A was the more pathogenic at all soil moistures; Gradus was more susceptible than Pilot throughout.

The failure of emergence in the controls at 85 % of saturation calls for comment. The quantity of water originally added to reach this figure was fully absorbed, but when moistened to this extent, the soil packed down with handling of the tins, so that it became

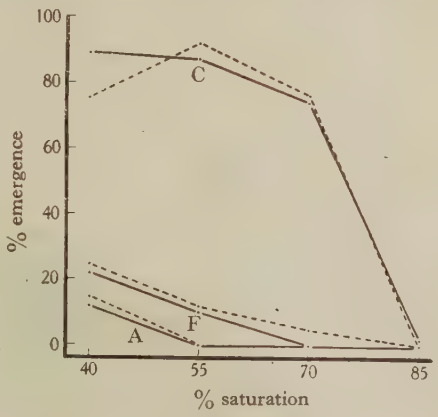
saturated. As, in the open field, soil moisture has not exceeded 65 %, it seems unlikely that physiological failure of the seed through excessive wetness of the soil has occurred there. Imperfect germination in the controls of other pot experiments was, however, probably due to this cause.

Two experiments were carried out in 1938 to determine the pathogenicity of *Pythium* isolates when added to *unsterilized* soil. One was carried out in pots, the other in drills in the open ground: in neither was there a marked or regular intensification of pre-emergence damping-off caused by the addition of *Pythium* inoculum. At the time of year (Jan.-Mar.) when these experiments were in progress the intensity of disease was very high in the field plots, so that a material increase of disease by additions of inoculum was hardly to be expected.

Though Sattar (1934), Padwick (1938) and Hull (1937) found that artificial inoculations of *Ascochyta Pisi* did not reduce emergence under greenhouse or laboratory conditions, it



Text-fig. 1. Percentage emergence of peas at different soil moistures. C, control; A. *Pythium*; F, *Pythium* F. Solid lines, temp. approx. 5° C.; broken lines, temp. approx. 12° C. Var. Pilot.



Text-fig. 2. Var. Gradus; otherwise as in Text-fig. 1.

was thought desirable to test the effect of this fungus in a very wet cold soil. The experiments were similar to the first one described with *Pythium*, the same soil and degree of soil moisture (80 %) being used. The containers were covered with glass and placed out of doors in January. The seed was thoroughly washed with sterile water and then dipped in strong spore suspensions of *Ascochyta* made from cultures 23 days old on quaker-oat agar. All but one of the isolates were newly obtained from Pilot seed. Four isolates of *Pleospora*, one a recent one, three of *Fusarium* and three of a *Pythium* of group D were also included in the experiment. As the *Pleospora* spored poorly, a disk from the edge of a young culture on potato-extract agar was placed beneath each seed. *Pythium* and *Fusarium*, being soil fungi, were inoculated into the soil in the usual way. Of the *Fusaria*, D and E were obtained from Pilot seedling axes and F from a cotyledon. They appeared to be distinct species. The results obtained are presented in Table 4.

Two of the *Fusarium*, two of the *Ascochyta* and all the *Pleospora* isolates did not reduce emergence: the remainder had some effect but were not in any way comparable with

Pythium. However, the application of the fungus to the outside of the seed is not equivalent to natural infection which often involves the cotyledons and seedling axis, and it is considered that *Ascochyta* and *Pleospora* were probably responsible for the emergence of Pilot seed being inferior to that of Gradus in 1937. In the experiment a parallel series of containers contained Gradus seed, but, apparently through the severity of the conditions, emergence was so poor in the controls that the results were abandoned.

An experiment in sterilized soil was conducted primarily to test the pathogenicity of the species of *Mucor* which had appeared so abundantly among the isolations from cotyledons in 1938 (Table 2). Two isolates from seedling axes and one from a cotyledon were used. Three forms of *Fusarium* (form B from a cotyledon, forms C and D from seedling axes) and three isolates of *Ascochyta Pisi* (two of which had been obtained from Gradus axes

TABLE 4. *Average emergence per container of six Pilot seeds*

Fungus	No. of replicates	Av. emergence
Control	21	4·5
Pythium D	9	0·2
Fusarium D	3	2·7
,, E	3	4·0
,, F	3	4·0
Pleospora	12	4·8
Ascochyta Pisi	3	4·3
,,	3	4·3
,,	3	2·7
,,	3	3·0
,,	3	1·3

TABLE 5. *Percentage emergence of peas*

Soil	Replicates	% emergence	
		Pilot	Gradus
Sterilized	9	83·7	68·2
Unsterilized	3	—	2·2
Sterilized + *Mucor* sp.	9	84·4	75·6
+ *Ascochyta Pisi*	9	76·5	60·0
+ *Fusarium* B	3	0·0	0·0
+ *Fusarium* C	3	86·7	71·1
+ *Fusarium* D	3	75·6	71·1

surface sterilized with silver nitrate) were also tested. The seed used was from the 1938 samples and was surface sterilized with mercuric chloride. The soil-borne fungi *Mucor* and *Fusarium* were inoculated into the soil in the manner previously adopted; with *Ascochyta* the seed was dipped in strong spore suspensions of the fungus. The soil moisture was kept at 60 % of saturation and the temperature maintained at 5–7° C. in an ice-chest. As a test of the suitability of the experimental conditions for the incidence of the disease, tins of unsterilized soil planted with Gradus seed were included. Three replicates were used, each involving fifteen seeds, but as the isolates of *Mucor* and *Ascochyta* did not differ among themselves a single figure is given for each species (see Table 5).

Of the isolates tested in this experiment only *Fusarium* B, which was very highly pathogenic, had any marked effect upon emergence, while the low emergence in unsterilized soil shows that the experimental conditions were suitable for a very high incidence of the disease.

The pathogenicity of every fungus which has occurred to a substantial extent among the isolations from diseased material has been tested, under conditions highly favourable for the disease, in one or more of the foregoing experiments, the results of which may be tabulated as follows:

Species	No. of isolates tested	Effect on emergence
Pythium spp.	18	Inhibited emergence almost completely
Fusarium spp.	6	One inhibited emergence, five had little or no effect
Mucor sp.	3	No effect
Ascochyta Pisi	8	Little or no effect
Pleospora sp.	4	No effect

It may therefore be concluded that the only fungi capable of causing the disease, which were isolated with any degree of frequency from the diseased embryos obtained from the field, were species of *Pythium*.

Summary

Mycological examination of pea seedlings which failed to emerge showed that attack often took place at a very early stage, often before germination had occurred. Various fungi were isolated from the cotyledons of diseased embryos; species of *Pythium* were obtained from almost every seedling axis. Of the isolates tested, species of *Pythium* and a species of *Fusarium* were the only ones capable of inhibiting emergence in sterilized soil, under conditions otherwise comparable with those of the field. The disease is accordingly attributed principally to species of *Pythium*. The species of *Pythium* concerned were conidia-bearing forms. Such of the isolates as developed sexual organs were referable to *P. de Baryanum* and *P. ultimum*.

I wish to express my indebtedness to Prof. W. Brown, F.R.S. for suggesting this problem and for general supervision of the work.

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EXPLANATION OF PLATE 10

Fig. 1. Effect of two isolates of *Pythium* on emergence of Pilot peas in sterilized soil at water contents ranging from 40 to 85 % of saturation. Top series, control; middle, *Pythium* A; bottom *Pythium* F.

Fig. 2. As Fig. 1 but with seed of var. Gradus.

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Fig. 2.

Fig. 1.

BAYLIS.—Fungi which cause Pre-emergence Injury to Garden Peas (pp. 210–18)

A COMPARATIVE STUDY OF STRAINS OF *RHIZOCTONIA SOLANI* (KUHN) WITH SPECIAL REFERENCE TO THEIR PARASITISM

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(With Plate 11 and 4 Text-figures)

During the past fifty years numerous plant diseases have been recorded as being caused by *Rhizoctonia Solani* Kuhn. Braun (1930) states that the fungus is able to attack 230 species of plants extending over sixty-six families. The question arose as to whether the species was on the whole omnivorous, or whether it was composed of a number of biological races of restricted host range. Peltier (1916) and Briton-Jones (1924) found little evidence of specialization, while Gratz (1925), Van der Meer (1926), Lauritzen (1929) and Le Clerg (1934) reported the existence of biological races, some at least of which are comparatively restricted in their pathogenicity. In particular there is strong evidence from several sources (Gratz, 1925; Van der Meer, 1926; Lauritzen, 1929; Wellman, 1932; Le Clerg, 1939; and Dennis, 1941) that strains occurring on potato and on crucifers are distinct from each other. Briton-Jones (1924) noted differences in the cultural behaviour of isolates, but not in their parasitism, and Dennis (1941) showed that isolates from potato were unable to cause rotting of swede roots. The investigation reported here arose in connexion with important damping-off diseases caused by *Rhizoctonia Solani* in certain vegetable crops, more particularly lettuce and various Brassicae.

Cross-inoculation studies

The following is a list of the isolates of *Rhizoctonia Solani* used, and of the hosts from which they were derived. The isolations were made by the writer unless otherwise stated:

Potato 1: damped-off potato stem from Spalding Marsh, Lincs.; by R. V. Tipler, June 1936.
Potato 2: sclerotia on tuber; Feb. 1937.
Potato 3: tuber showing jelly-end rot, Sleaford, Lincs.; Oct. 1937.
Tomato 1: strain obtained from Cheshunt; 1933.
Tomato 2: damped-off seedling, Slough; Abdel-Salem, 1930.
Sea-kale: diseased leaf, Slough; Nov. 1936.
Radish: obtained from G. H. Pethybridge, reisolated from its host; Feb. 1937.
Stock: damped-off seedling, Slough; June 1936.
Lettuce 1, Lettuce 2: damped-off seedlings, Slough; Nov. 1936.
Zinnia: damped-off seedling, Slough; June 1936.
Pea: cotyledon of pea which failed to germinate in field, Slough; by G. T. S. Baylis, Apr. 1936.
Cotton: damped-off seedling, Nanking, China; S. C. Shen, Mar. 1936.
Beet, grass, endive: cultures from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Hyphal tip cultures were prepared from each isolate in the first instance. The cultures were maintained on 2 % malt agar and renewed every 4 weeks. The various isolates were added to the soil in the following standardized manner. Slough garden soil at about 30 % of its water-holding capacity, as determined by the method of Keen & Raczkowski (1921), was passed through a 3 mm. sieve and sterilized in bulk by autoclaving for 3½ hr. at 15 lb. pressure. It was then allowed to stand for at least 10 days before use. The inoculum was taken from a 12-days-old culture (25° C.) on a medium consisting of 98 % sand +2 % cornmeal, and was added at the rate of 5 g. of inoculum per 100 g. of

air-dried soil. The proportion of organic matter added to the soil was therefore less than one part in one thousand. The inoculated soil was then apportioned between the various containers, seeded or planted, and its moisture content raised to 60 % of saturation, which was found in preliminary trials to be most suitable for the work. Throughout the experiment the soil moisture was maintained constant by the appropriate additions of distilled water. No elaborate attempt was made to regulate the temperature of the greenhouse, but thermograph records were kept. In the control series sterilized soil without the addition of organic matter was used. In view of the varied nature of the host plants used, no uniform method of assessing the intensity of disease was practicable.

Potato

Tubers of var. Eclipse, Kerr's Pink and Great Scot were dipped in 1:183 mercuric chloride for 1½ hr. to kill any viable sclerotia present on the surface and then allowed to sprout. When the sprouts were about ½ in. long their number was reduced to 4–5 for each tuber, and the tubers were planted with 3 in. of inoculated or sterilized soil above their upper surface. The experiment was duplicated at each of three temperatures. After 3½ weeks the tubers and sprouts were washed clean and graded into the following three classes:

I. No lesions present on the sprouts.
II. Lesions present on shoots but less than 3 cm. in length.
III. Lesions present on shoots greater than 3 cm. in length.

TABLE 1. *Pathogenicity of isolates of* Rhizoctonia Solani *to potato*

	Av. temp. 24·0° C. No. of tubers in class			Av. temp. 19·5° C. No. of tubers in class			Av. temp. 16·5° C. No. of tubers in class		
Isolate	I	II	III	I	II	III	I	II	III
Control	6	0	0	6	0	0	6	0	0
Potato 1	3	3	0	1	3	2	2	4	0
,, 2	4	1	1	2	1	3	0	1	5
Tomato 1	1	1	4	0	0	6	1	5	0
Seakale, radish, stock, lettuce 1	6	0	0	6	0	0	6	0	0

TABLE 2. *Pathogenicity of isolates of* Rhizoctonia Solani *to tomato*

Isolate	Av. % emergence	Av. % of emerged plants which damped-off	Av. % of emerged plants which showed stem lesion
Control 1	87·5	0	0
Potato 1	91·5	25·1	15·0
,, 2	90·0	12·5	1·1
,, 3	92·0	8·1	3·8
Tomato 1	85·0	9·4	2·3
,, 2	87·5	10·2	6·8
Cotton	42·5	37·6	0
Grass	68·5	32·8	5·8
Seakale, radish, stock, pea, beet	89·5–90·5	0	0

The experiment with var. Eclipse is recorded in Table 1. Similar results were obtained with the other two varieties. At the three temperatures used only the strains from potato and tomato produced attack. There was a definite suggestion that one at least of the strains from potato was most active at the lowest temperature, whereas the strain from tomato was most active at the higher temperature.

Tomato

Forty seeds of var. Kondine Red were sown in each pot. The experiment was replicated five times for each isolate and was maintained at approximately 18–22° C. The intensity of infection was determined by the amount of pre- and post-emergence damping-off and by the number of plants bearing stem lesions 6 weeks after the date of sowing. The results (Table 2) show that the only isolates which

attacked tomato were those derived from potato, tomato, cotton and grass. Strains from various crucifers, pea, beet, and as found in another experiment not tabulated, from lettuce, zinnia and endive did not attack tomato seedlings.

Seakale (Crambe maritima)

Rooted and budded seakale cuttings were planted in sterilized soil which had been inoculated with the various strains of *Rhizoctonia Solani*. Prior to planting the cuttings were well washed and scraped to reduce the chance of accidental infection. The experiment was run at two temperature levels, averaging 15·2 and 21·6° C. respectively. After 3 weeks the soil was washed away and the plants were graded into four classes according to the nature of the injury shown.

I. Plant healthy with one or more buds developing on the crown.
II. Original buds attacked but the crowns were able to develop secondary buds.
III. All buds damped-off but no rotting of the crown occurred.
IV. Similar to III but with rotting of the crown.

The results (Table 3 and Pl. 11, fig. 1) are typical examples of plants from the high temperature series, and show clearly the selectivity to seakale of strains from crucifers. They suggest also that the intensity of attack is greater at the higher temperature, and that the isolate from stock is less virulent than those from seakale and radish.

TABLE 3. *Pathogenicity of isolates of* Rhizoctonia Solani *to seakale*

	Av. temp. 15·2° C.				Av. temp. 21·6° C.			
Isolate	I	II	III	IV	I	II	III	IV
Control	10	0	0	0	9	1	0	0
Seakale	0	0	4	6	0	0	0	10
Radish	0	3	3	4	0	1	0	9
Stock	0	6	4	0	0	3	4	3
Potato 1, potato 2, tomato 1, lettuce 1	10	0	0	0	10	0	0	0

TABLE 4. *Pathogenicity of isolates of* Rhizoctonia Solani *to swede, radish and stock*

Isolate	Swede % emergence in control-inoc. soil S.E.	Radish % emergence in control-inoc. soil S.E.	Stock % emergence in control-inoc. soil S.E.
Seakale	86·5 ± 1·92	64·0 ± 4·9	69·5 ± 1·3
Radish	86·0 ± 1·93	63·5 ± 4·9	69·0 ± 1·4
Stock	86·0 ± 1·93	62·5 ± 4·9	67·5 ± 1·5
Cotton	55·2 ± 3·7	—	—
Grass	44·8 ± 4·0	—	—
Endive	24·0 ± 3·3	—	—

Swede

The various isolates were tested both on mature swede roots and on seedlings.

(*a*) *Swede roots.* Swede roots 3–4 in. diam. were inoculated by the method of Granger & Horne (1924) with cultures grown on 2 % malt agar, maintained for 14 days in a moist chamber at 17–20° C., after which the amount of rotted tissue was determined. The strains from seakale, radish and stock gave respectively 6·9, 6·6 and 2·9 g. of rotted tissue. Strains from potato (1 and 2), tomato 1 and lettuce gave no attack.

(*b*) *Seedling infection.* Table 4 shows the average increase or decrease in emergence from inoculated as compared with sterilized soil, the data being based upon a tenfold replication, with sixty seeds sown per pot. The results are also illustrated in part in Pl. 11, fig. 2. Similar data for radish and stock seedlings are included in Table 4. Differences greater than three times the standard error are regarded as significant, and only those differences giving such a result are listed in Table 4. Isolates derived from cotton, grass and endive are capable of attacking swede seedlings as well as those derived from crucifers, which were equally pathogenic to swede, radish and stock. There was no evidence to indicate the existence of any specialization amongst the isolates derived from cruciferous host plants. Isolates derived from potatoes 1, 2 and 3, tomatoes 1 and 2, lettuces 1 and 2, zinnia, pea and beet gave non-significant differences.

Lettuce

Seventy seeds of var. Trocadero were sown in each pot and the virulence of the various strains was determined by the amount of post-emergence damping-off, each treatment being replicated ten times. The isolates capable of causing post-emergence damping-off were those derived from lettuce, zinnia, and one from tomato.

TABLE 5. *Pathogenicity of isolates of* Rhizoctonia Solani *to lettuce*

Isolate	Av. % post-emergence damping-off
Control	0·0
Lettuce 1	29·7
,, 2	25·6
Zinnia	15·2
Tomato 2	20·8
Tomato 1, potato 1, 2, 3, seakale, radish, stock, pea, cotton, beet, grass, endive	0·0

TABLE 6. *Summary of cross-inoculation experiments*

Isolate	Potato	Tomato	Seakale	Swede roots	Swede seedlings	Radish seedlings	Stock seedlings	Lettuce seedlings
Potato 1	+	+	−	−	−	−	−	−
,, 2	+	+	−	−	−	−	−	−
,, 3	.	+	.	.	−	.	.	−
Tomato 1	+	+	−	−	−	−	−	−
,, 2	.	+	.	.	−	.	.	+
Seakale	−	−	+	+	+	+	+	−
Radish	−	−	+	+	+	+	+	−
Stock	−	−	+	+	+	+	+	−
Lettuce 1	−	−	−	−	−	−	−	+
,, 2	.	−	.	.	−	.	.	+
Zinnia	.	−	.	.	−	.	.	+
Endive	.	−	.	.	+	.	.	−
Beet	.	−	.	.	−	.	.	−
Cotton	.	+	.	.	+	.	.	−
Grass	.	+	.	.	+	.	.	−

+ = attack. − = no attack.

The results described in the above series of cross-inoculation experiments are collecte in Table 6, from which the following conclusions can be drawn.

(1) Some strains of *Rhizoctonia Solani* exhibited a very restricted host range whilst othe did not exhibit such a marked degree of specialization.

(2) The isolates from potato confined their attack to solanaceous host plants, and tl strain tomato 1 behaved in a similar manner. The strain tomato 2, on the other hand, w able to attack lettuce as well as tomato. This latter result agrees with that obtained b Abdel-Salem (1933).

(3) Isolates derived from cruciferous plants were confined in their attack to these host There was a sharp distinction between solanaceous and cruciferous isolates, and this is i agreement with the results of Gratz (1925), Van de Meer (1926), Lauritzen (1929) an Dennis (1941).

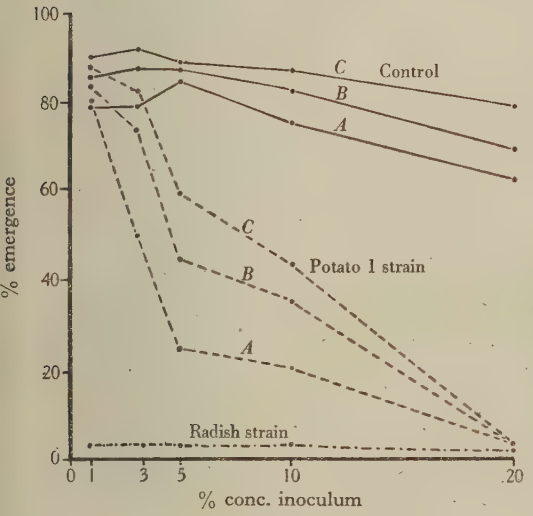
(4) Composite isolates derived from lettuce and zinnia were confined in their attack t these hosts. The endive strain, on the other hand, only attacked swede seedlings, but nc lettuce. This strain, obtained from Baarn, is probably the same as was used by Schult (1937), who found that it parasitized a considerable range of plants including lettuce and

various crucifers. Schultz's strain was isolated in Java in 1922, and it is therefore not impossible that its reactions have become altered by long-continued culture.

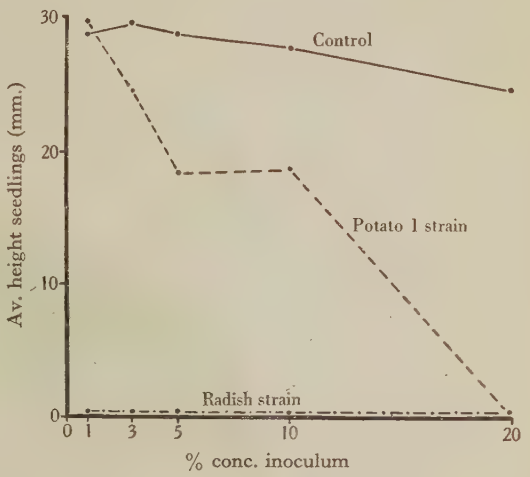
(5) The strain derived from beet was unable to attack any of the plants used and those derived from cotton and grass are interesting in that they are capable of attacking both cruciferous and solanaceous host plants.

Effect of amount of inoculum on experimental results

The foregoing results were obtained by a technique which involved the addition to the soil of not more than 0·1 % of additional organic matter (p. 219). Wiant (1929), Samuel & Garrett (1932), Hynes (1937), Le Clerg (1934) and Newton & Mayers (1935*a*) used as inocula cultures of the organism on sterilized wheat grains. Unfortunately, the amount of



Text-fig. 1. Effect of concentration of inoculum on the emergence of swedes in soil infected with *Rhizoctonia Solani*. *A*, after 7 days; *B*, after 14 days; *C*, after 21 days.



Text-fig. 2. Effect of concentration of inoculum on the growth of swedes in soil infected with *Rhizoctoni Solani*.

organic material added was not always stated, but it is clear that in some cases it was considerable. Thus Schultz (1937) added 'several 2 sq. cm. pieces, cut from Petri-dish cultures on potato-extract agar or beer-wort agar', to each flower pot (approx. 4 in.), and he noted the abundant growth of hyphae not merely upon the surface of the soil but throughout its interior. One must presume therefore that very unnatural conditions were set up in his experiments. Because of discrepancy in the results obtained by various workers it was important to determine whether the amount of inoculum used had any material effect on the type of result obtained.

In the following experiments two strains, potato 1 and radish, were used. When tested by the method described above, the former was non-parasitic upon swede seedlings whereas the latter caused severe pre-emergence damping-off. Inocula of sterilized wheat grains on which the two strains had grown for 10 days were incorporated with the soil at the rate of 1, 3, 5, 10 and 20 g./100 g. of air-dried soil. The various preparations were potted and swede seeds sown at the rate of 40 per pot. The soil was maintained at 60 % of its water-holding capacity. Controls were set up with the addition of

uninoculated wheat grains. The effect of amount of inoculum on emergence of the seedlings after 7, 14 and 21 days is given in Text-fig. 1, and Text-fig 2 shows the effect on the height (from base of hypocotyl to tip of youngest leaf) of the seedlings after 21 days growth. A typical series of pots is illustrated in Pl. 11, fig. 3. At the end of the experiment the seeds in pots which contained the highest amount of inoculum of the potato strain and which had failed to germinate were dug up. Samples were germinated on moist filter paper in Petri dishes and compared with ordinary commercial seed: the latter gave 96·6 % germination and the former 93·3 %.

Thus the effect of the potato strain upon swede seeds in the presence of a high organic content of the soil is an inhibition of germination and not a true parasitic attack. With the radish strain at all concentrations of inoculum used no trace of the seeds could be found at the end of the experiment. The inhibition of germination and the stunting effect upon the growth of swede seedlings which are brought about by the potato strain in the presence of a large amount of inoculum indicates that chemical secretions of the fungus come into play. Similar effects have been described by Mannozzi (1932), who showed that an extract from a $1\frac{1}{2}$-months-old culture of *Rhizoctonia* retarded the growth of wheat seedlings, and Newton & Mayers (1935*b*), who found that an extract from a strain from potato markedly reduced the growth of turnip and carrot seedlings. The latter authors suggest that this toxic effect might be used to evaluate the relative immunity of plant species towards the fungus. This would appear to be highly doubtful in view of the artificial conditions under which the toxic action is shown, i.e. in the presence of abnormal amounts of organic material added to the soil.

The above results with varied amounts of inoculum probably offer an explanation of the somewhat divergent results which various workers have obtained in cross-inoculation studies. Unfortunately, the amount of inoculum added has not always been stated, but it is clear that if it had been large there would be a serious risk of mistaking an artificially produced chemical effect for true parasitism. It is suggested therefore that the host range of some strains of *R. Solani* is narrower than has appeared from the results obtained by certain experimental methods.

Certain physiological aspects

Part of the object of this investigation was to determine whether strains of *R. Solani* were suitable subjects for an attempted physiological analysis of parasitism. Certain results were distinct and promising, and of these a brief account is given. In this work the two strains potato 1 and radish were used throughout.

Cuticular penetration. The behaviour of the two isolates on forced seakale leaves and potato sprouts was investigated, the main object being to determine whether both strains were capable of penetrating both hosts, and if so to determine their subsequent progress. Small disks of inoculum were placed on the surface of forced seakale leaves or potato sprouts in moist glass dishes, and examined after 24–48 hr., to see if penetration had occurred. A convenient method of detecting the latter was to strip off the epidermis and examine under the microscope. The hyphae in some cases were stained with lactophenol and cotton blue, and serial sections were also cut. Material for the latter was fixed in Carnoy's fluid and stained by Stoughton's method (1930). The following account is based on the results obtained by all three methods:

Radish strain on seakale leaves: ready penetration occurred at a large number of points. The fungus rapidly invaded the tissue which was rotted, the mycelium was mainly intercellular.

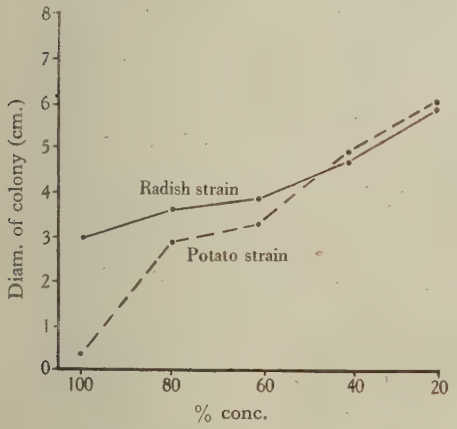
Potato strain on seakale leaves: appressoria formed and penetration was only occasional; the lesions were minute and non-progressive.

Radish strain on potato sprouts: minute brown lesions were produced, but no penetration was detected.

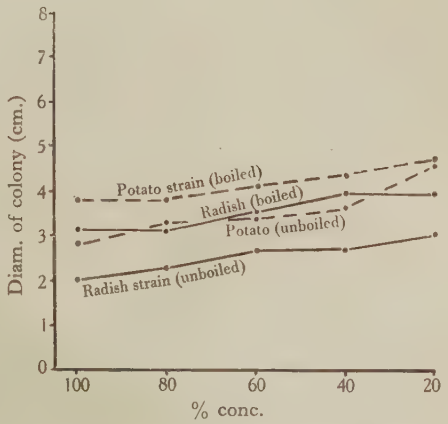
Potato strain on potato sprouts: progressive rotting occurred with intercellular hyphae.

Similar results as regards invasion or non-invasion of tissue were obtained when the cuticle had been stripped off beforehand, so that, while there is some evidence that each strain does not readily penetrate the inappropriate host, the factor which determines attack or non-attack is an internal one.

Growth of isolates in extracts of hosts. An extract of swedes was prepared by freezing minced root tissue for 12 hr. and after thawing expressing the juice in a hand press. Various dilutions of this extract were set up, passed through a Seitz filter and placed in 20 c.c. lots in sterile Petri dishes. Small disks of inoculum of the potato 1 and radish strains were then placed in the middle of the dish, each strain being replicated three times at each concentration. Text-fig. 3 gives the average diameter of the colonies produced in 4 days at 24° C.



Text-fig. 3. Growth of *Rhizoctonia Solani* in various concentrations of swede extract



Text-fig. 4. Growth of *Rhizoctonia Solani* in various concentrations of potato extract

TABLE 7. *Growth of* Rhizoctonia Solani *in swede extract*

Isolate	Av. diam. of colony (cm.) after keeping extract: 0 days	4 days
Radish	3·0	3·8
Potato 1	0·5	4·3

Both strains are retarded in linear growth by increasing concentration of the extract. Over the range 20–80 % the two strains respond similarly, but with further increase of concentration the potato strain is strongly affected. In turnip extract of full strength it is almost completely inhibited, no growth being visible for the first 3 days. With the radish strain in full strength swede extract growth was visible within 12 hr. from the beginning of the experiment.

The repressive effect of swede extracts upon the growth of the potato strain of *Rhizoctonia* disappears on ageing of the extract. Table 7 gives the diameter of cultures made in freshly prepared extract and in the same after it had been held for 4 days in a refrigerator. On the other hand, a short period (1 min.) of boiling the freshly prepared extract has no appreciable effect on the properties of the extract.

Text-fig. 4 illustrates in a similar way the growth of the potato and radish strains on potato extract over a range of concentrations of the latter. In this case boiling of the extracts increases the growth rate of both strains, and though the potato strain grows better than the radish strain under the same conditions, there is not the distinct repressive effect which was shown with undiluted swede juice. Thus while it might be suggested that the inability of the potato strain to attack swedes is associated

with intolerance to the sap of the plant, the like explanation could not readily be applied to the failure of the radish strain to attack potato sprouts.

Secretion of pectinase enzyme. This was examined by the technique of Brown and co-workers (see Brown, 1936). Cultures were made on autoclaved plugs of potato tuber and swede-root tissue, and extracts prepared after various intervals. While it was found that each strain (potato 1 and radish) gave the most active extracts when grown on plugs of the appropriate host, the major effect was that the maximum enzymic activity was produced by the potato strain in a much shorter time (5 as against 15 days) than by the radish strain, and that this effect held whether it was grown on plugs of potato or swede.

Previous work referred to by Brown (1936) showed that the enzymes of *Bacillus carotovorus*, *Pythium* and *Botrytis* are more active on turgid tissue and that a loss in turgor slows down the enzymic activity of the *Bacillus carotovorus* and *Pythium* enzymes whilst the enzyme of *Botrytis* may be completely inhibited. Table 8 gives the results of a similar experiment carried out with two strains of *Rhizoctonia*, which are in full agreement with the earlier work.

TABLE 8. *Enzymic activity of extracts*

	Av. wt. of rotted tissue (g.)			
	Potato plugs		Swede plugs	
Extract	Subturgid	Turgid	Subturgid	Turgid
Potato 1: On potato plug	0·60	1·19	0·26	0·71
On swede plug	0·52	0·95	0·25	0·54
Radish: On potato plug	0·34	0·47	0·17	0·34
On swede plug	0·64	0·67	0·43	0·98

In relation to the preceding type of experiment the behaviour of the two isolates of the fungus was determined when small pieces of agar inoculum of the fungus were placed on blocks of turgid and subturgid swede tissue. Potato tuber tissue was also tried but was found to be unattacked by either strain under the conditions of the experiment although the fungus grew quite well on the surface.* The striking result obtained was that injection with water rendered swede tissue much less susceptible to invasion by the crucifer strain of *Rhizoctonia*. Thus in one experiment the weight of rotted tissue was reduced to one-tenth by injection with water. This result was repeatedly confirmed.

It was noted that when normal swede tissue, i.e. with air present in the intercellular spaces, was attacked by the crucifer strain of *Rhizoctonia*, the rotted part developed a dark brown colour and little superficial mycelium was present. Water-soaked tissue, on the other hand, showed much superficial mycelium, and a slight amount of rotting on the surface, especially round the disk of inoculum. There was no brown discoloration and no evidence that the hyphae penetrated to any extent into the interior.

The increased resistance to *R. Solani* which injection produces in swede tissue stands in pointed contrast to the behaviour of potato tissue to various organisms (Brown, 1936) and of tomato to various saprophytic and parasitic bacteria (Johnson, 1937). Both these authors found that injection with water distinctly increased susceptibility. The cause of this divergence cannot be determined at the moment, though it might be suggested that in the case of *R. Solani* on injected swede tissue failure to attack arises from the anaerobic conditions which are set up within the tissue.

* The failure of the potato strain to attack potato tubers is remarkable in view of the fact that potato sprouts are readily attacked. Shapovalov (1922) showed that under certain conditions *Rhizoctonia Solani* was capable of causing a rotting of the stem end of elongated tubers. Another type of injury has been recorded by Ramsey (1917), where the fungus caused symptoms similar to scab, the rot extending as a dry core into the tuber. Potato tubers showing both types of injury have been obtained and *R. Solani* has been isolated. However, on inoculating this fungus into potato tubers, only the second type of injury was produced. The rate of attack was very slow, and after about 3 weeks only a small amount of tissue had been decomposed.

Summary of results

A series of cross inoculation experiments was carried out with isolates of *Rhizoctonia Solani* derived from various host plants. The existence of marked biologic races is indicated, some strains having a wide host range whilst others exhibit a more selective parasitism. Divergent results were obtained with seedling hosts when an inoculum of the fungus of high organic content was used. This was shown to be due to the unfavourable soil conditions created by the fungus. An attempt was made to investigate the factors underlying the pronounced selective parasitism exhibited by some of the isolates, in particular the potato 1 and radish. The behaviour of the two isolates on susceptible and non-susceptible hosts was examined. The resistance of crucifers to the isolates from potato appeared to be correlated with the presence of a substance which was inhibitory to the growth of the fungus, whilst the appropriate strain was able to tolerate it. Enzyme was found to be produced on susceptible and non-susceptible host tissue, and differences in the secretion of the pectinase by the two strains was noted. The behaviour of subturgid and turgid swede tissue to the action of the fungus and enzyme was shown to differ. The enzyme from the cruciferous isolate was most active on turgid tissue whilst the action of the fungus was almost completely inhibited.

The writer wishes to express his thanks to Prof. W. Brown for much advice and assistance during the progress of this work.

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EXPLANATION OF PLATE 11

Fig. 1. Effect of various isolates of *Rhizoctonia Solani* on seakale cuttings. Left to right, isolates derived from seakale, radish, stock, tomato, lettuce, potato 2, potato 1; control.

Fig. 2. Effect of various isolates of *Rhizoctonia Solani* on the germination and growth of swedes.

Fig. 3. Effect of concentration of inoculum on the germination and growth of swedes. A, control, with sterilized wheat grains added. B, inoculated, with the potato 1 isolate of *Rhizoctonia Solani* growing on sterilized wheat grains.

(*Received* 11 *February* 1941)

Fig. 1.

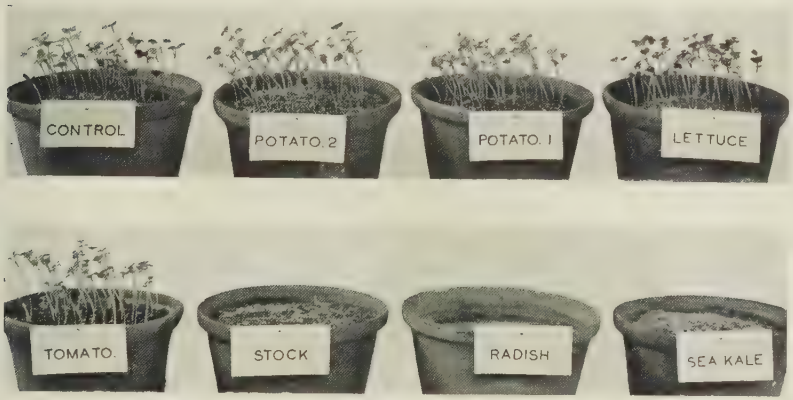

Fig. 2.

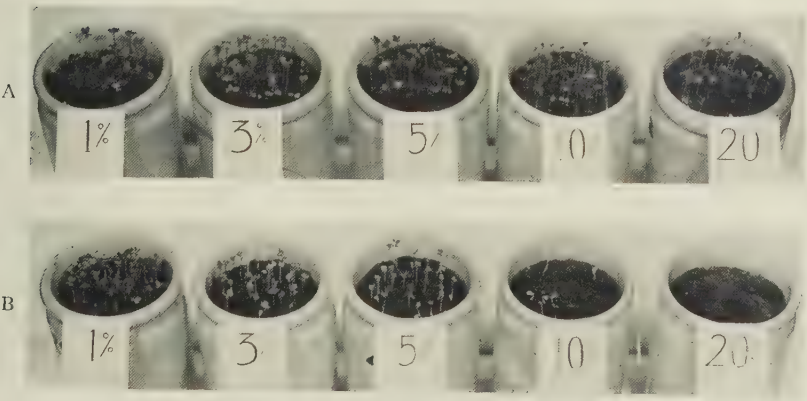

Fig. 3.

STOREY—A Comparative Study of Strains of *Rhizoctonia Solani* (Kuhn) with Special Reference to their Parasitism (pp. 219–28)

STUDIES IN THE PHYSIOLOGY OF THE VIRUS DISEASES OF THE POTATO

IV. A COMPARISON OF THE NITROGEN RELATIONS OF HEALTHY AND CRINKLE POTATOES; TOGETHER WITH SOME OBSERVATIONS ON THE NITROGEN RELATIONS OF A "CARRIER" VARIETY

By E. BARTON-WRIGHT

(With 7 Text-figures)

Introduction

Barton-Wright & McBain (1932) showed that potato plants infected with leaf-roll differed markedly in their carbohydrate metabolism from healthy plants, whereas in their nitrogen relations (1933*b*) no such striking differences could be discovered, with the exception that the total nitrogen content of all parts of the diseased plants was lower than in healthy material. An examination of the carbohydrate relations (1933*a*) between healthy potato plants and those suffering from the compound disease known commonly as 'crinkle' gave results which were not statistically significantly different for reducing sugars, sucrose and starch, except towards the end of the growing season, but against this must be set the fact that the diseased plants matured earlier than the healthy. Minor differences were discovered in the behaviour of the carbohydrates between healthy and diseased material, but these were not of such a nature as to account for the serious loss in yield from this disease. These observations were in the main confirmed by Cockerham (1939) for virus *X* (one of the components of crinkle) in potatoes. It was also shown by Barton-Wright & McBain (1933*a*) that the presence of a latent virus in a variety, in this case 'paracrinkle' (Salaman & Le Pelley, 1930) does not cause significant differences in the carbohydrate variations of the 'carrier' variety. Since the incidence of crinkle in a potato crop causes such a heavy loss in ware, it is clear that this fall in yield must be sought elsewhere than in any derangement of the carbohydrate metabolism. A comparison was therefore made of the nitrogen relations of healthy and crinkle-infected potato plants, in order to determine whether any derangement of the nitrogen metabolism of these plants might account for the loss in yield.

Experimental

(1) *Material.* The same material was used as in the carbohydrate work on this subject (Barton-Wright & McBain, 1933*a*). The variety Arran Victory was infected with virus $A+X$ to give Murphy's crinkle by grafting scions of diseased Irish Chieftain on to healthy plants, and paracrinkle was transmitted to the carrier variety, President, by grafting scions of King Edward to this variety. All plants were grown in pots in insect-proof greenhouses.

(2) *Duration of experiments.* The various nitrogen fractions, as well as total nitrogen, were estimated over 24 hr. periods at different times in the growing season; the samples of leaves and petioles for these diurnal experiments being gathered at 2 hr. intervals. All diurnal determinations began at 10 p.m. G.M.T. Seasonal observations were also made on the healthy and crinkle plants by taking samples at weekly intervals over the growing season.

(3) *Grading.* Grading was carried out as in the previous work (1933*b*). Fifty healthy and fifty diseased plants were used for each diurnal series.

(4) *Preparation of material.* After each collection, the material was treated and worked up in exactly the same way as previously described (1933*b*). The estimations made were: total nitrogen, including nitrate nitrogen, total crystalloid nitrogen, ammonia nitrogen, amino-acid nitrogen, amide nitrogen, nitrate nitrogen and asparagine nitrogen.

Since in these diurnal and seasonal fluctuations definite chemical substances were concerned and not merely amide-N and amino-N, the results are expressed as follows:

(i) *Asparagine* N. The assumption was made that the whole of the amide N is present in the form of amides of the asparagine type.

(ii) *Amino-acid* N. This was obtained by deducting ammonia N and amino-N due to amide N from the total amino-N recorded by the formol method.

(iii) *Residual* N. This term is used to cover the fraction of crystalloid N not accounted for by the sum of the asparagine N, amino-acid N, nitrate N and ammonia N.

(iv) *Protein* N. This value was obtained by deducting crystalloid N (non-protein N) from the total nitrogen figure.

(v) *Expression of results.* All results were calculated as a percentage of the residual dry weight, except where otherwise stated. The seasonal determinations have been calculated as a percentage of the total nitrogen.

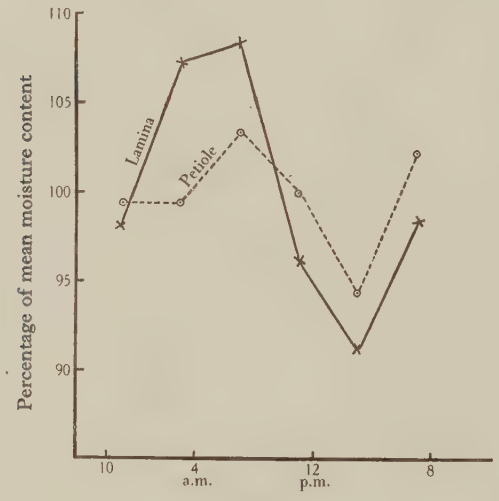

Fig. 1

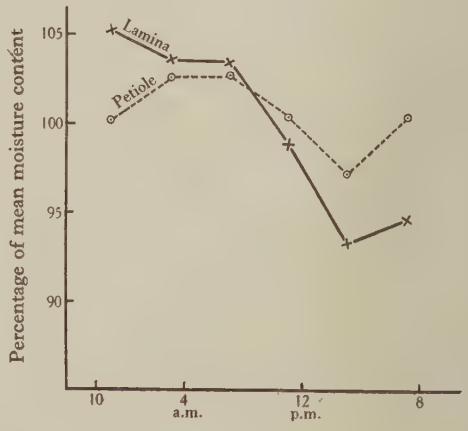

Fig. 2

Fig. 1. Variations in the moisture content of laminae and petioles of healthy Arran Victory plants (diurnal series I). Moisture content calculated as grams moisture per 100 g. residual dry weight and shown as percentages of the mean for laminae and petioles. The points that have been plotted are the means of successive pairs of observations.

Fig. 2. Variations in the moisture content of laminae and petioles of crinkle Arran Victory plants (diurnal series I). Moisture content calculated as in healthy material and means of successive pairs of observations plotted in the curves.

Experimental observations

Two sets of diurnal observations were carried out on healthy and crinkle Arran Victory plants and two upon healthy and paracrinkle-carrying President plants.

Diurnal variations in healthy and crinkle Arran Victory. Series I (24 *Apr.* 1933)

Moisture changes. Figs. 1 and 2 show the diurnal trend of water content of lamina and petiole of healthy and diseased plants. In the healthy plants there is an accumulation of water by night and a fall by day. This diurnal trend is by no means so marked in the diseased material (Fig. 2). The difference in water content between healthy and diseased laminae is

strongly significant. The significance of the difference of the means calculated by the elaborated 'Student's' method given by Fisher (1936) is:

$S(x-x')$	t	P	
65·7	3·236	<1·0 %	Strongly significant

Nitrogen fluctuations in healthy and crinkle Arran Victory

Table 1 gives the values for the total N recorded for the first set of diurnal observations in laminae and petioles of healthy and diseased material. The total nitrogen figures for both laminae and petioles of the diseased plants are higher than in the healthy, and this difference is statistically significant.

TABLE 1. *Observations on the total N of healthy and diseased laminae and petioles of Arran Victory*

	Healthy		Crinkle	
Time	Laminae %	Petioles %	Laminae %	Petioles %
10 p.m.	5·222	3·139	6·485	3·451
12 „	5·327	2·356	6·068	3·001
2 a.m.	5·799	2·468	6·278	3·049
4 „	5·751	2·667	6·834	3·877
6 „	5·582	2·847	6·166	3·398
8 „	5·725	2·393	5·712	3·258
10 „	5·380	2·630	6·549	3·674
12 „	6·002	2·554	6·343	3·091
2 p.m.	6·387	2·885	6·232	3·047
4 „	5·168	2·324	6·454	3·359
6 „	5·091	2·422	6·115	3·464
8 „	5·339	2·640	6·287	2·900
Mean	5·564	2·610	6·293	3·297

TABLE 2. *Significance of difference of means for values of total N between healthy and diseased laminae and healthy and diseased petioles*

	$S(x-x')$	t	P	
Laminae	0·729	5·318	<1·0 %	Strongly significant
Petioles	0·687	6·190	<1·0 %	„ „

A similar result has been recorded by Cockerham (1939) for the laminae of plants suffering from virus *X*, in which he found that the values for total nitrogen were significantly higher in the diseased plants. Not only is the total nitrogen content of laminae and petioles of diseased material higher than that of healthy, but this also applies to other parts of crinkle-infected plants, as is shown by the figures given below:

	Total N as % of residual dry weight	
	Healthy	Crinkle
Lamina	5·639	6·931
Midrib	4·078	5·571
Petiole	2·768	3·490
Upper stem	1·799	2·296
Lower stem	1·396	1·671

It is clear that there are marked differences between the total nitrogen content of healthy and diseased material.

Table 3 gives the values for the significant differences of the means of the various nitrogen fractions of the laminae. Of these six nitrogen fractions, three, i.e. asparagine N, residual N and protein N, are significantly different in healthy and diseased material. It should be noted in this connexion that residual N is probably concerned in the transport of nitrogenous substances in the plant, while protein is of the greatest importance in nitrogen metabolism.

TABLE 3. *Significance of difference of means of values for different nitrogen fractions in healthy and diseased laminae*

	$S(x-x')$	t	P	
Ammonia N	0·0018	0·6764	50–60 %	Not significant
Amide N	0·0449	2·8220	1·0–2·0 %	Significant
Amino-acid N	0·0233	1·9870	5–10 %	Not significant
Nitrate N	0·0071	0·5040	60–70 %	,,
Residual N	0·3316	10·0700	< 1·0 %	Strongly significant
Protein N	0·3430	2·7690	1·0–2·0 %	Significant

The corresponding figures for the nitrogen fractions of healthy and diseased petioles are given in Table 4. Thus, in the petiolar nitrogen fractions significant differences are also to be found. In this case nitrate nitrogen, unlike the lamina fraction, shows a significant difference.

TABLE 4. *Significance of difference of means for values of different nitrogen fractions in healthy and diseased petioles*

	$S(x-x')$	t	P	
Ammonia N	0·0069	1·8580	5–10 %	Not significant
Amide N	0·0809	4·1580	< 1 %	Strongly significant
Amino-acid N	0·0047	0·3251	60–70 %	Not significant
Nitrate N	0·2588	3·1940	< 1·0 %	Strongly significant
Residual N	0·0426	0·5590	50–60 %	Not significant
Protein N	0·3240	4·2960	< 1·0 %	Strongly significant

Diurnal variations in healthy and crinkle Arran Victory. Series II (4 *June* 1933)

Moisture changes. Figs. 3 and 4 show the fluctuations in water content of laminae and petioles of healthy and diseased material for the second series of diurnal observations that were made. There is, as in series I, a rise in water content in the lamina of the leaf during the night and a fall during the day in both healthy and diseased material, but the fluctuations in moisture in the diseased laminae and petioles, unlike the healthy, are not so well marked, and the fall in water content of the diseased material is only of short duration compared with the healthy.

The difference of the means between healthy and diseased laminae of water content as in series I is significant:

$S(x-x')$	t	P	
38·0	2·397	2–5 %	Significant

Diurnal variations in nitrogen. The values for the total nitrogen content of laminae and petioles of the second diurnal series of observations on Arran Victory are given in Table 5 which shows (*a*) that the total nitrogen content in both healthy and diseased material has fallen from the figures obtained in series I and (*b*) that the total nitrogen content of the

diseased material still shows a higher value than that of the healthy. The difference of the means are strongly significant:

TABLE 5. *Observations on the total N of healthy and diseased laminae and petioles of Arran Victory*

	Healthy		Crinkle	
Time	Laminae %	Petioles %	Laminae %	Petioles %
10 p.m.	3·971	1·089	4·804	1·579
12 ,,	3·730	1·095	4·567	1·643
2 a.m.	4·135	1·089	4·699	1·522
4 ,,	4·060	1·131	4·829	1·819
6 ,,	3·906	1·108	4·872	1·375
8 ,,	4·145	0·953	4·650	1·348
10 ,,	4·029	1·191	4·934	1·485
12 ,,	3·846	1·006	4·858	1·711
2 p.m.	3·945	1·084	4·923	1·700
4 ,,	4·050	1·117	4·592	1·748
6 ,,	3·697	1·212	4·518	1·501
8 ,,	3·758	1·093	4·629	1·477
Mean	3·933	1·097	4·740	1·576

TABLE 6. *Significance of difference of means of values for total N between healthy and diseased laminae and healthy and diseased petioles*

	$S(x-x')$	t	P	
Laminae	0·807	12·330	< 1·0 %	Strongly significant
Petioles	0·479	9·636	< 1·0 %	,, ,,

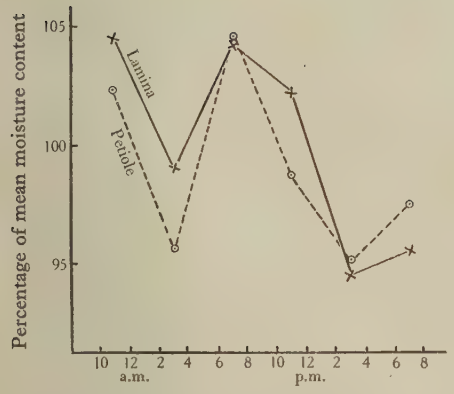

Fig. 3. Variations in the moisture content of laminae and petioles of healthy Arran Victory (diurnal series II).

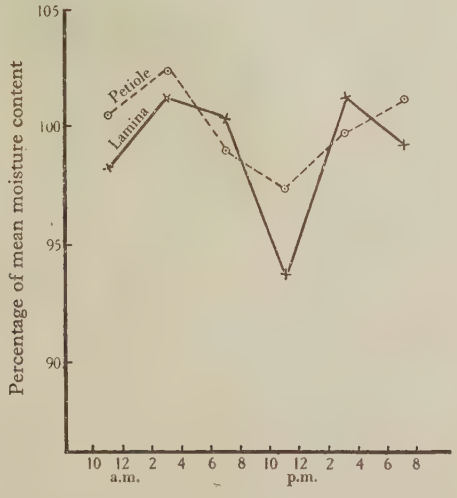

Fig. 4. Variations in the moisture content of laminae and petioles of crinkle Arran Victory (diurnal series II).

Table 7 gives the values for the significant difference of means for the various nitrogen fractions of the laminae. As in series I, amide N, residual N and protein N are all significantly different and in addition nitrate N is also now significantly different.

TABLE 7. *Significance of difference of means of values for different nitrogen fractions in healthy and diseased laminae*

	$S(x-x')$	t	P	
Ammonia N	0·0050	1·228	20–30 %	Not significant
Amide N	0·0433	3·660	<1·0 %	Strongly significant
Amino-acid N	0·0010	0·098	>90 %	Not significant
Nitrate N	0·0349	4·036	<1·0 %	Strongly significant
Residual N	0·1171	5·391	<1·0 %	,, ,,
Protein N	0·6110	10·250	<1·0 %	,, ,,

Seasonal variations in healthy and crinkle Arran Victory

Figs. 5–7 show the seasonal variations in the more important nitrogen fractions, i.e. protein N, residual N and nitrate N, in healthy and diseased laminae. In the healthy laminae there is a sharp rise in protein N for the first 3 weeks of the growing season, and thereafter the protein N remains relatively constant, whereas in the crinkle laminae protein N remains relatively constant and immobile throughout the growing season. Residual N (Fig. 6) in the healthy laminae at first shows a sharp fall and then remains relatively constant, whereas in the diseased material, like protein N, it remains relatively constant and immobile throughout the growing season. The fluctuations in nitrate N (Fig. 7) in both healthy and diseased material follow a very similar trend.

Nitrogen fluctuations in the laminae of healthy plants and plants carrying paracrinkle

Two sets of diurnal observations were carried out on the nitrogen fluctuations in healthy President laminae and in the laminae of President plants carrying paracrinkle. The significance of the difference of the means is given for total nitrogen as well as for the remaining nitrogen fractions in Tables 8 and 9.

TABLE 8. *Significance of difference of means for total N and other nitrogen fractions in laminae of healthy and paracrinkle-carrying President. Series I* (8 *May* 1933)

	$S(x-x')$	t	P	
Total N	0·1960	1·9210	5–10 %	Not significant
Ammonia N	0·0052	0·4831	60–70 %	,,
Amide N	0·0053	0·5344	50–60 %	,,
Amino-acid N	0·0340	1·2110	20–30 %	,,
Nitrate N	0·0224	1·3410	10–20 %	,,
Residual N	0·0800	1·6320	10–20 %	,,
Protein N	0·2600	1·8410	5–10 %	,,

TABLE 9. *Significance of difference of means for total N and other nitrogen fractions in laminae of healthy and paracrinkle-carrying President. Series II* (12 *June* 1933)

	$S(x-x')$	t	P	
Total N	0·0400	0·6667	50–60 %	Not significant
Ammonia N	0·0027	0·2182	80–90 %	,,
Amide N	0·0051	0·4811	60–70 %	,,
Amino-acid N	0·0078	0·6324	50–60 %	,,
Nitrate N	0·0039	0·9721	30–40 %	,,
Residual N	0·0409	1·3410	20–30 %	,,
Protein N	0·0250	1·4820	20–30 %	,,

It is clear that there are no significant differences between total nitrogen or any of the remaining nitrogen fractions in healthy and carrier plants, and that the presence of a latent virus does not apparently disturb the nitrogen relations of a carrier variety.

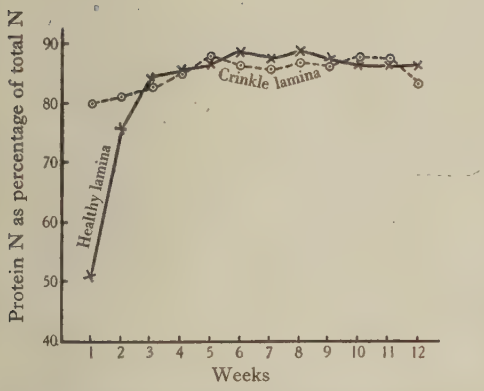

Fig. 5. Seasonal fluctuations in protein N in the laminae of healthy and crinkle Arran Victory. Samples gathered at weekly intervals and results expressed as a percentage of total nitrogen.

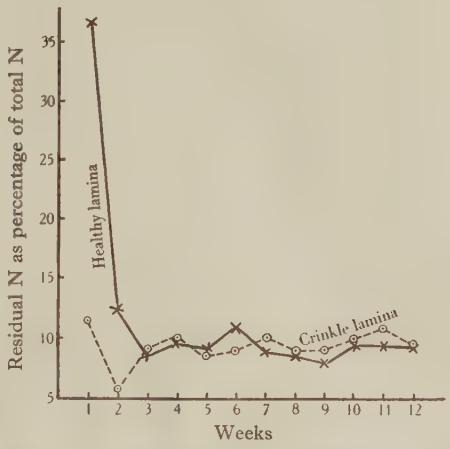

Fig. 6. Seasonal fluctuations in residual N in the laminae of healthy and crinkle Arran Victory. Samples gathered at weekly intervals and results expressed as a percentage of total nitrogen.

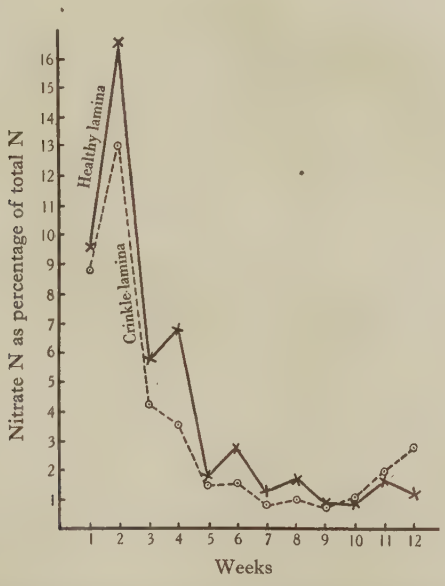

Fig. 7. Seasonal fluctuations in nitrate N in laminae of healthy and crinkle Arran Victory. Samples gathered at weekly intervals and results expressed as percentage of total nitrogen.

Discussion

The fact that significant differences were found in total nitrogen, as well as in the more important nitrogen fractions between healthy and crinkle infected plants, points very strongly to the view that the presence of this disease in potato plants leads to a derangement of their nitrogen metabolism. It is possible, therefore, that the heavy loss in yield that always accompanies crinkle in a potato crop is due to some at present unknown disturbance in the nitrogen metabolism of the affected plants. It has already been shown (Barton-Wright & McBain, 1933*a*) that there are only minor differences to be found in the distribution and fluctuations of carbohydrates in healthy and crinkle material, so that the heavy loss in crop yield cannot reasonably be assigned to a derangement of carbohydrate metabolism. Such differences as were discovered in carbohydrate behaviour are probably secondary phenomena following upon a primary disturbance in some other direction, notably as suggested here in the nitrogen metabolism.

Cockerham (1939) confirmed the work of Barton-Wright & McBain (1933*a*) that there are no gross differences in the carbohydrate behaviour of healthy and virus *X* infected potatoes. Nevertheless, he states that the small differences he was able to detect might possibly account for the loss of ware, which is surprising because he was able to find large significant differences in total nitrogen content between healthy and diseased plants.

If the suggestion put forward here be correct, namely, that crinkle causes derangement of the nitrogen metabolism of the plants, then the metabolic changes produced by leaf-roll and crinkle are fundamentally different. In plants suffering from leaf-roll the carbohydrate metabolism is profoundly disturbed (Barton-Wright & McBain, 1932) and normal translocation of sugars to the tubers seriously dislocated. In crinkle, on the other hand, the carbohydrate metabolism is apparently only affected in very minor directions and translocation of sugars to the tubers not seriously disturbed, yet there is a heavy fall in yield. The data available at present suggest that this fall in yield is in some at present unknown way correlated with changes in nitrogen metabolism.

Finally, the presence of a latent virus in a carrier variety does not appear to disturb either the carbohydrate or the nitrogen metabolism of the infected plants, and there is no significant difference in yield. In the circumstances, therefore, the presence of a latent virus in a variety is harmless from the point of view of crop yield.

Summary

An investigation was made into the nitrogen relations of healthy and crinkle infected (virus $A+X$) potato plants. Statistically significant differences were found in total nitrogen, protein nitrogen, residual nitrogen, amide nitrogen, and towards the close of the growing season in nitrate nitrogen between the laminae of healthy and diseased plants. Similar statistically significant differences were found in the petioles. The total nitrogen content and protein nitrogen content of all parts of crinkle infected plants at all times in the growing season were found to be higher than in healthy material, and this difference is statistically significant. It is suggested that the loss in yield brought about by crinkle is due to some at present unknown derangement of the nitrogen metabolism of the diseased plants. The presence of a latent virus in a carrier variety, in this case paracrinkle in the variety President, does not cause any significant differences in total nitrogen or any of the nitrogen fractions.

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TRANSMISSION OF TOBACCO ETCH VIRUSES BY APHIDES

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The fact that severe etch virus (S.E.V.) is transmitted by *Myzus persicae* Sulz. has already been recorded (Kassanis, 1939). This paper gives the results of more detailed studies on the relationships between tobacco etch viruses and their vectors.

Most of the work was done with S.E.V. obtained from Dr W. M. Stanley, but comparative tests were made with the related mild etch virus (M.E.V.) supplied by Dr W. C. Price. The conditions of the insectary and methods of handling and culturing the aphides were similar to those described by Watson (1936, 1938). The aphides were raised on turnips and radishes, both of which are immune to the etch viruses. Leaves of medium size, cut from tobacco plants infected for at least 14 days, were used for the infective feeding, and young tobacco seedlings were used as test plants. The first symptom appears 6–8 days after infection and usually not on all plants in the same day. The time probably depends on the part of the leaf in which the insect was feeding, so that the virus has to travel different distances to reach the central vein. Examination of the leaves infected by single aphides, by the iodine method, shows a retention of starch at the point of infection.

Preliminary tests showed that aphides, fed for only 2 min. on an infected leaf, and transferred immediately to test plants, could infect them even if removed after 2 min. Thus the whole process of acquiring the virus and infecting a healthy plant can occur within 5 min., showing that there is no delay in transmission such as occurs with some viruses. These tests also showed that infection could be obtained readily with a single aphis, and in all the experiments described, unless otherwise stated, only one aphis per plant was used. The phrase 'percentage of infection' is the number of plants out of 100 infected by single aphides.

In addition to *M. persicae* it was found that S.E.V. is transmitted by *M. circumflexus* (Buckt.), *Aphis rhamni* (Boyer), *Aphis fabae* (Scop.) and *Macrosiphum gei* (Koch.).

Experimental

Effect of variations in the length of preliminary starving and infective feeding times

Watson (1938) showed that the percentage of infection with Hyoscyamus virus 3 was greatly increased if aphides were starved before getting their infective feeding, provided the time of infective feeding was also short. Similar results have been obtained with both S.E.V. and M.E.V. Table 1 shows the summarized results of 11 experiments with S.E.V. In each experiment forty-five plants were used, five each for nine different treatments. The treatments consisted of variations of from 0 to 4 hr. in the starving time before infective feeding and from 2 min. to 1 hr. for the infective feeding time.

Increasing the preliminary starving time increases the percentage of infection. This effect disappears if the infective feeding time is itself increased, so that with an infective feeding time of 1 hr. the previously starved insects transmit no better than those receiving no preliminary starving period. The longer the time that aphides fed on the source of the virus the less likely were they to transmit, and when aphides that had been raised on infected tobacco plants were transferred to healthy plants the percentage of infection was very small. The most infections with single aphides occur when they have not fed for at least 4 hr. and are then fed for only a few minutes on the infected plant.

Table 2 shows the summarized results of four replications of an experiment made to compare the transmission of M.E.V. by *M. persicae* with that of S.E.V. There were six treatments for each virus, and a total of thirty-five aphides was used for each treatment. The treatments consisted of a preliminary starving period of either 0 or 4 hr., combined with an infective feeding time of 2 min., 1 hr. or 4 hr.

M.E.V. reacts to variations in preliminary treatment and in the length of the infective feeding exactly like S.E.V., and the two viruses are equally readily transmitted. This differs from results obtained by Watson (1939) with mild and severe strains of Hyoscyamus virus 3. The severe strain gave a higher percentage of infection, which Watson correlated with the higher virus content of sap infected with this strain. The virus content of sap from plants with S.E.V. is also considerably higher than that from plants with M.E.V. (Bawden & Kassanis, 1941), yet these two are transmitted to the same extent.

TABLE 1. *Effect of variations in the length of preliminary starving and infective feeding times*

Preliminary starving time	Infective feeding time			Total	% of infection
	2 min.	15 min.	1 hr.		
None	6	5	1	12	7·2
1 hr.	22	11	3	36	21·8
4 hr.	32	7	1	40	24·2
Total	60	23	5	88	
% of infection	36·3	13·9	3·0		

TABLE 2. *Comparative transmission of* S.E.V. *and* M.E.V.

Preliminary starving time	Strain of virus	Infective feeding time			Total	% of infection
		2 min.	1 hr.	2 hr.		
None	S.E.V.	2	0	—	2	5
	M.E.V.	3	0	—	3	8
4 hr.	S.E.V.	16	0	0	16	45
	M.E.V.	15	1	0	16	45

TABLE 3. *Effect of post-infection starving time*

Preliminary starving time	Post-infection starving time				
	None	15 min.	1 hr.	3 hr.	6 hr.
None	14 %	0	0	0	0
4 hr.	52 %	49 %	34 %	17 %	0

Effect of variations in treatment after the infective feeding

Preliminary tests showed that not only was no incubation period necessary before the aphides could transmit S.E.V. but that they failed to transmit unless they were placed on test plants soon after removal from the source of infection. Table 3 shows the summarized results of ten replications of an experiment made to determine how long *M. persicae* retained its infectivity after removal from the source of infection. In each replication fifty plants were used, five for each of the ten treatments. The treatments consisted of preliminary starving of either 0 or 4 hr., and variations in the period before being placed on the test plants of from 0 to 6 hr. All the aphides received an infective feeding period of 2 min.

The results again show the effect of preliminary starving in increasing the percentage of infection, and also that the insects treated in this way retain their infectivity longer. Even these, however, had completely lost their ability to infect healthy plants after 3 hr.

It seemed possible that temperature might affect the length of time for which the aphides remained infective, and a comparison was therefore made with aphides kept at 3° C. after being fed for 2 min. on the infective plant. This experiment was repeated eight times. On each occasion 5 plants were used for each treatment, and the percentage of infection obtained is given in Table 4. These results can be contrasted with those of Table 3 obtained at a temperature of about 20° C.

Here again the beneficial effects of a preliminary starving in increasing the length of time for which the aphides remain infective are shown, and also those of lowering the temperature. The effect of temperature may partly explain the variation in percentage of infection obtained at different seasons. Aphides, under optimal conditions for transmission, in the months of June–August gave 46 % whereas in the colder months of October–December they gave 59 %, although growing conditions for both plants and insects were less favourable.

TABLE 4. *Effect of post-infection starving time at* 3° C.

Preliminary starving time	Post-infection starving time								
	None	15 min.	1 hr.	2 hr.	3 hr.	4 hr.	16 hr.	20 hr.	24 hr.
None	12 %	15 %	10 %	2 %	5 %	—	—	—	—
4 hr.	—	37 %	—	—	—	22 %	20 %	10 %	7 %

Watson & Roberts (1939) have shown that individuals of *M. persicae* remain infective with Hyoscyamus virus 3 longer if they are starved after their infective feeding than if they feed continuously. Aphides infective with S.E.V. behave similarly. Eight replications of an experiment were made in which insects, first starved for 4 hr. and then given an infective feeding period of 2 min., were allowed to feed for varying periods on a healthy plant before being transferred to the test seedlings. The percentage of infection obtained with insects left on the healthy plant for 2, 5, 15 and 30 min. was 35, 35, 2 and 0 % respectively. Thus, in contrast to the 3 hr. for which insects starved at room temperature remained infective, those feeding continuously lost most of their infectivity in 15 min. and all of it in 30 min.

As the insects lose their infectivity so quickly, it is obvious that in natural conditions it must be unusual for one to infect more than one plant. To determine whether insects could infect more than one plant without further access to a source of infection, the following experiment was repeated three times, using 10 aphides each time. The aphides were given a preliminary starving period of 4 hr., fed for 2 min. on the source of infection, and then transferred to the first series of test plants. After 2–3 min. they were transferred to the second series of plants on which they were also allowed to feed for 2–3 min. They were given similar short feeding periods on a third and fourth series of plants and left overnight on a fifth. Seven of the aphides failed to infect any of the test plants and the performances of the other twenty-three are recorded individually in Table 5, infected tests plants being shown by a +.

Only one aphis succeeded in infecting four plants and two aphides infected three plants. More infections occurred in the first set of test plants than in any other, but the total obtained

in the last four sets was greater than that in the first. One of the most striking results is that an aphis may fail to infect from one to four test plants on which it feeds and then infect the next.

It seemed possible that aphides which had once become infective and then lost their infectivity might behave differently from other insects. To test this possibility the same aphides were used in an identical experiment done on consecutive days. Five aphides were used in each experiment and the experiment was repeated five times. The aphides were starved for 4 hr., fed for 2 min. on an infective plant and then transferred singly to healthy test plants, on which they were allowed to remain overnight. The insects were then again starved, given an infective feeding of 2 min. and transferred to the second test plants.

TABLE 5. *Consecutive feeding on a series of five plants*

Consecutive healthy plants					Total of infected plants
A	B	C	D	E	
o	+	o	o	o	1
+	o	o	o	o	1
o	+	o	+	o	2
o	+	o	o	+	2
o	o	o	o	+	1
+	o	o	o	+	2
+	+	o	o	o	2
+	o	o	o	+	2
+	o	o	o	+	2
+	+	+	o	o	3
o	+	o	o	+	2
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+	o	o	o	o	1
+	o	+	o	o	2
+	o	o	o	o	1
+	o	o	o	o	1
+	o	+	o	o	2
+	+	+	o	o	3
+	o	o	o	o	1
+	+	o	+	+	4
Total 18	8	5	2	8	41

The total infections obtained on the first days were nineteen and on the second twenty-four. The records of the individual aphides showed that some transmitted on the first day but not on the second, some on the second but not on the first, and some on both days. The fact that an aphis transmitted on one day but not on another suggests that the failure to get 100 % infection in transmission tests with single aphides is not because the culture of aphides used contains individuals unable to transmit, but because of some other unknown factor. The fact that some aphides transmitted on both days also shows that having once been infective has no effect on future ability to transmit.

Transmission from virus mixtures

Aphides, given varying lengths of preliminary starving and infective feeding on plants infected with S.E.V. and tobacco mosaic virus, when transferred to healthy test plants have always no infections or infections with S.E.V. alone.

Bawden & Kassanis (1941) showed that when S.E.V. is inoculated to tobacco plants with Hyoscyamus virus 3, the latter appears to be suppressed and replaced by S.E.V., for sap taken from young leaves of such plants reacts only with antiserum to S.E.V. Similarly, the young leaves of plants inoculated with a mixture of the two viruses react serologically only with S.E.V. antiserum. In an attempt to get a more sensitive test for Hyoscyamus virus 3 than the serological one, aphides were fed on plants rubbed with a mixed inoculum, and then transferred to healthy tobacco plants. Of the 130 test plants, ten developed symptoms typical of infection with Hyoscyamus virus 3 and thirty-three those of S.E.V. In this experiment no distinction was made between aphides fed on leaves actually rubbed with the inoculum and those fed on leaves with systemic infection. A second test was therefore made with 200 aphides, 100 of which were fed on leaves rubbed with a mixed inoculum of S.E.V. and Hyoscyamus virus 3 and the other 100 on young leaves of these plants showing systemic symptoms. The results, given in Table 6, show that the aphides were able to recover Hyoscyamus virus 3 alone from the rubbed leaves but not from the others. It seems that in the rubbed leaves both viruses multiply, but that in those that become infected by systemic spread S.E.V. suppresses Hyoscyamus virus 3 almost completely. From leaves infected systemically with Hyoscyamus virus 3 alone, at least 50 plants would have been

TABLE 6. *Transmission from plants inoculated with* S.E.V. *and Hyoscyamus virus* 3

	Insects fed on rubbed leaves	Insects fed on leaves with systemic infection
Insects infected with Hyoscyamus virus 3	16	0
Insects infected with S.E.V.	39	49
Total	55	49

infected by the aphides under the conditions of the experiment, and the fact that from these leaves none was infected is strong evidence that they contained little or no Hyoscyamus virus 3.

DISCUSSION

The results obtained with S.E.V. are very similar to those obtained by Watson & Roberts (1939) with Hyoscyamus virus 3, potato virus *Y* and cucumber virus I. The percentage of infection with all is increased greatly by starving insects before feeding them on the source of infection and is greatly reduced if the period of feeding on the source of infection is increased. The only difference in the behaviour of S.E.V. and Hyoscyamus virus 3 is that aphides lose their infectivity even more rapidly with S.E.V. than with the latter, and consequently fewer plants can be infected in succession by one aphid. As with Hyoscyamus virus 3, the time for which aphides remain infective is greater when they are fasting than when they are feeding.

Some workers, e.g. Doolittle & Walker (1928), suggest that vectors which rapidly lose their infectivity act purely mechanically, their stylets merely behaving as needles which become contaminated with virus while feeding on infected plants. The results described here, however, are difficult to fit to this hypothesis. If it were true, it would hardly be expected that aphides fed on plants infected with both S.E.V. and tobacco mosaic virus would regularly transmit only S.E.V., for the concentration of tobacco mosaic virus in the

sap is at least 100 times that of S.E.V., and it is transmitted mechanically much more easily. Nor would it be expected that infective insects fed successively on a series of healthy plants could fail to infect early ones and then infect later ones in the series. This fact suggests that the aphis is ejecting virus discontinuously and not merely having it absorbed by the plant from the outside of the stylets. Again, the theory of mechanical transmission cannot explain the great increase in the percentage of infectivity obtained by using starved insects given a short infective feeding.

The results with S.E.V. support the suggestion of Watson (1938) that the aphides ingest the virus and that in the aphis the virus comes into contact with something that inactivates it. Her earlier suggestion (1936) that the inactivation resulted from specific antibody formation seems unlikely, for inactivation with S.E.V. would seem to occur too quickly for antibody formation to be possible, and the fact that aphides which have lost their infectivity are as active as vectors as other aphides also tells against the view that they acquire any antibodies against the virus. Her later suggestion that the virus is inactivated by something that is secreted in greater quantity by aphides while feeding than while fasting, e.g. a proteolytic enzyme, would explain all the observed effects with S.E.V.

Summary

Severe etch virus is transmitted by *Myzus persicae*, *M. circumflexus*, *Aphis rhamni*, *A. fabae* and *M. gei*. Although the content of mild etch virus in sap is much less than that of S.E.V., both are transmitted to the same extent by *M. persicae*. The percentage of infection using single aphides is greatest with aphides that are starved for 4 hr. or more and then fed on the source of infection for 2 min. Continuous feeding on healthy plants or diseased plants greatly reduces the efficiency of the vector. The length of time for which aphides remain infective is also increased from 15 min. to a few hours if the aphides are starved instead of allowed to feed; it is also greatly increased in starved insects if they are kept at low temperature. Provided the feeding time on each test plant is small, one aphis may infect up to four plants.

I wish to thank Mrs M. A. Watson for much helpful advice.

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STUDIES OF THE BIOLOGY OF THE DEATH-WATCH BEETLE, *XESTOBIUM RUFOVILLOSUM* DE G.

IV. THE EFFECT OF TYPE AND EXTENT OF FUNGAL DECAY IN TIMBER UPON THE RATE OF DEVELOPMENT OF THE INSECT

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(With 2 Text-figures)

It has been shown (Fisher, 1940) that timber attacked by wood-destroying fungi is more suitable for the development of the death-watch beetle than sound or mould-infested wood, and it was suggested that an increase in the extent of decay tends to decrease the duration of the life cycle. Further work on this fungus-insect relationship has been carried out, and the effect of fungal decay on the nutritional value of the wood to the larva has been studied in co-operation with the Wood Chemistry Section of this Laboratory. Samples of timber, decayed to varying degrees by fungi producing decay of the white and brown rot types, were submitted to infestation by the death-watch beetle, and a comparison made by chemical analysis of the composition of the decayed wood before and after attack by the insect. The technique adopted in the preparation of the material, and the biological observations recorded in the course of the experiments form the subject of the present paper: the chemical aspect of part of the investigation has been published separately (Campbell & Bryant, 1940). The prolonged life cycle of *Xestobium rufovillosum* has necessitated the continuation of the biological experiments over a period of years, 1935–40, but results as complete as possible are now presented.

Experimental

Material and methods

The rate of development of *Xestobium* has been followed in English oak sapwood (*Quercus robur*) and in cricket bat willow (*Salix fragilis*), decayed to varying degrees by *Phellinus cryptarum* Karst. and *Polystictus versicolor* (Linn.) Fr., both producing white rots, and experiments have also been undertaken with English oak sapwood decayed by *Coniophora cerebella* Pers., a brown rot. The wood used was selected from sound timber and cut into samples measuring $6 \times 1\frac{1}{2} \times 1\frac{1}{2}$ in. for decaying and subsequent attack by the insect. After initial weighing the samples were sterilized in an autoclave and inoculated with the species of fungus under investigation. Decay was allowed to proceed while samples were removed at intervals in order to obtain a series representative of different degrees of decay. The initial oven-dry weight of each sample was estimated at the commencement of the experiment from the average moisture content of controls typical of the series as a whole. After varying periods of exposure to decay, the superficial growth of fungal mycelium was removed from the samples and their oven-dry weight determined. The loss in oven-dry weight due to fungal attack, expressed as a percentage of the original dry weight of the sound wood, was taken as a measure of the extent to which the sample had been decayed by the fungus concerned.

The technique then used for exposing samples to insect attack was similar throughout the experiments. The decayed samples were arranged in groups of approximately equal loss in weight due to decay, with the object of exposing each group separately to infestation by *Xestobium rufovillosum*, and to compare the rate of larval development and the duration of the life cycle of the insect in each. At the same time, one decayed sample representative of the average loss in weight of each group was reserved as a control for chemical anslysis, and was not exposed to insect attack.

Except in the case of samples decayed by *Coniophora cerebella*, it was necessary to roughen the surfaces of the samples in order to facilitate egg laying, and to render them suitable for entry by first stage larvae, since previous observations on the habits of the insect had shown that smooth surfaces were unsuitable for attack. An attempt was made to limit the initial insect population in each group by controlling the number of eggs laid per sample. A small disk $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{4}$ in. was cut from one end of each sample and exposed to beetles in a separate vessel for egg laying at 20° C. and 86 % relative humidity, the conditions under which the experiments were conducted throughout and which earlier work had shown were suitable for the development of the insect (Fisher, 1938). When approximately thirty eggs had been laid on each disk, these were attached to their respective samples in their original position, and each group normally of four samples was then confined in a tin with muslin cover, and kept under the above conditions of temperature and humidity, insect attack being allowed to continue undisturbed for varying periods before an examination was made to determine its rate of progress. A group of control samples received identical treatment, whilst in addition a set of controls, not exposed to insect attack, was provided for the chemical part of the investigation.

Results

At the outset, the experiments were planned for the dual purpose of giving information on the duration of the life cycle of the insect in timber in different stages of decay and also of providing a sufficient quantity of larval frass of known origin for chemical analysis. It was not, therefore, possible to obtain from these experiments additional data which examination of the results subsequently showed would have been of interest. For instance, information upon the comparative weights of insects reared for a given period in samples of different degree of decay would have been of value, but the primary object of the work and the slow rate of larval development in slightly decayed wood made this impossible. Sufficient information has, however, been obtained to give a general indication of the effect of fungal decay on the duration of the life cycle under stated conditions of temperature and humidity.

The samples were not examined in detail to note the progress of attack until the appearance of an exit-hole determined the minimum duration of the life cycle. In different samples within the same group the first exit-holes sometimes appeared at widely separated intervals, with the result that detailed examination of all samples in a group was not invariably carried out at the same time. In some instances, particularly when emergence had been recorded from some samples in a group but not in others, the final examination was made before the appearance of exit-holes. When each sample was cut up, records were kept of the stage of development of all the insects found, alive or dead, and the condition of the sample with regard to distribution and severity of attack was noted. The frass and the unattacked remains of the wood in each group were then stored for chemical analysis and comparison.

A summary of the observations and results obtained is given in Tables 1, 2 and 4.

A. *White rots*

(*a*) *Oak sapwood decayed by* Phellinus cryptarum (*Table* 1).

The limitations of the experiment have introduced factors which affect the results and are the most probable explanation of the variation in individual samples within each group. Of these factors, non-uniformity of distribution of decay within a sample of known average loss in weight due to fungal attack is of particular importance, as it may have affected the rate of larval boring and development in different parts of a sample. Moreover, in examining the results, each group of four samples should be considered as a whole, because the wandering habit of the newly emerged larvae may have led to their concentration on individual samples or parts of samples most suitable for attack.

Despite these limitations, however, certain definite conclusions can be drawn. In the first place, it is apparent that sound undecayed wood (group F) is unsuitable for infestation; although the eggs laid on the samples in this group hatched, the first stage larvae did not succeed in boring and attack therefore did not develop. In contrast to this, infestation took

TABLE 1. *Duration of life cycle in oak sapwood decayed by* Phellinus cryptarum: *at* 22–25° C. *and* 80–90 % *relative humidity* (*equivalent moisture content* 18–20 %)

Group and sample	Loss in wt. by decay %	Duration of attack and final condition of sample	Larvae* recovered: Alive	Dead	Pupated	Minimum duration of life cycle (months)
A 6	16·0	54 months: a few tunnels throughout; powdered in patches	2 (*b*) 2 (*c*)	4	1	52–54 (emerged)
13	16·2	65 months: attack moderate throughout; not severely powdered	1 (*a*) 4 (*b*) 1 (*c*)	2	1	< 65 (not emerged)
3	16·4	61 months: attack moderate but severely powdered in parts	1 (*a*) 2 (*b*) 1 (*c*)	—	1	54–61 (emerged)
26	22·1	54 months: attack moderate throughout	1 (*a*) 3 (*b*)	3	1	< 54 (not emerged)
	Av. loss in wt. = 17·7	Total insects recovered = 31	Av. minimum life cycle = 55 (emerged)			
B 11	22·7	45 months: attack slight; a few patches of more severely powdered wood	—	4	—	—
38	26·2	44 months: attack extensive but not completely powdered	1 (*a*) 5 (*b*) 2 (*c*)	4	2	30–36 (emerged)
10	26·5	44 months: attack poor, tunnels few and scattered	—	4	1	36–38 (not emerged)
34	27·1	36 months: severely powdered, very little unattacked wood left	23 (*a*)	—	—	—
	Av. loss in wt. = 25·6	Total insects recovered = 46 + 2 (on floor of vessel)	Av. minimum life cycle = 33 (emerged)			
C 1	29·5	40 months: attack very slight, died out early; sample abnormal	1 (*c*)	—	—	—
21	33·6	30 months: completely powdered except for small part containing living larva and beetles	1 (*a*)	13	2	< 30 (not emerged)
29	34·0	40 months: attack extensive but unattacked wood still present	10 (*b*) 2 (*c*)	6	2	(< 40: beetles dead, not emerged)
22	34·1	30 months: comparable with sample 21, but less severely powdered and unattacked wood present	14 (*b*)	—	2	< 30 (not emerged)
	Av. loss in wt. = 32·8	Total insects recovered = 53	Av. minimum life cycle = < 30 (not emerged)			
D 31	35·9	21 months: attack very severe, almost completely disintegrated	25 (*a*) 12 (*b*) 2 (*c*)	—	6	< 21 (not emerged
30	36·1	21 months: comparable with sample 31	44 (*a*)	—	—	—
18	36·4	21 months: attack severe but some unattacked wood present	16 (*a*) 3 (*b*) 1 (*c*)	—	1	20 (emerged)
41	37·5	21 months: attack slight	3 (*a*) 9 (*b*) 4 (*c*)	—	1	< 21 (not emerged)
	Av. loss in wt. = 36·5	Total insects recovered = 127	Av. minimum life cycle = 20 (emerged)			

E	36	38·6	25 months: attack moderate throughout	1 (*a*) 8 (*b*) 15 (*c*)	3	1	< 25 (not emerged)
	9	38·9	25 months: attack severe, almost completely powdered	4 (*a*)	20	7	25 (emerged)
	37	40·6	25 months: attacked throughout, completely powdered in parts	8 (*a*) 3 (*b*) 1 (*c*)	1	2	15–20 (emerged)
	8	41·9	25 months: attack moderate throughout, severe in parts	9 (*b*, *c*)	6	2	23–25 (emerged)
		Av. loss in wt. = 40·0	Total insects recovered = 91	Av. minimum life cycle = 22 (emerged)			
F	1	Controls.	62 months: no traces of attack in any of the samples	—	—	—	—
	2	No decay					
	3						
	4						
			Total insects recovered = nil				
G	66	49·6	12 months: samples bound together after 6 months; attack severe, almost completely powdered	36 (*b*)	48	7	< 12 (1 emerged, 6 not emerged)
	55	49·9					
	76	51·4					
	56	51·5					
		Av. loss in wt. = 50·6	Total insects recovered = 91	Av. minimum life cycle = < 12 (emerged)			
H	57	59·0	12 months: attack severe, reduced to powder	12 (*b*)	—	10	< 12 (not emerged)
	69	61·8					
	58	57·3	21 months: attack slight after 12 months; 12 larvae transferred from other samples; attack finally severe but not completely powdered	12 (*b*)	18	20	16 (emerged)
	61	61·5					
		Av. loss in wt. = 59·9	Total insects recovered = 60	Av. minimum life cycle = < 12 (not emerged)			
J	70	73·5	6 months: attack severe, disintegrated in parts but not completely powdered	2 (*b*) 45 (*c*)	—	—	—
	53	70·0	12 months: samples bound together after 6 months; attack severe, disintegrated and reduced to dust	—	76	3	< 12 (1 emerged, 2 not emerged)
	71	74·8					
	65	75·0					
		Av. loss in wt. = 73·3	Total insects recovered = 126	Av. minimum life cycle = < 12 (emerged)			

* Size: (*a*) $\frac{3}{4}$ to full grown; (*b*) $\frac{1}{2}$ to $\frac{3}{4}$ grown; (*c*) < $\frac{1}{2}$ grown.

TABLE 2. *Duration of life cycle in willow decayed by* Polystictus versicolor: *at* 22–25° C. *and* 80–90 % *relative humidity (equivalent moisture content* 18–20 %). *Sapwood and heartwood not distinguished*

Group and sample	Loss in wt. by decay %	Duration of attack and final condition of sample	Larvae* recovered			Minimum duration of life cycle to emergence (months)
			Alive	Dead	Pupated	
A 65	9·7	42 months: attack moderate and largely confined to decayed strip on edge; distribution of decay not uniform	1 (*b*) 2 (*c*)	—	—	
58	9·7	42 months: decay and attack confined to one face; condition similar to above but attack slightly more severe	2 (*a*) 1 (*c*)	5	—	—
40	12·2	53 months: decay and attack confined to edge; attack slight; heartwood present	2 (*b*) 2 (*c*)	3	—	—
53	12·9	53 months: attack slight, chiefly confined to edge; heartwood present	2 (*b*) 2 (*c*)	1	—	—
	Av. loss in wt. = 11·1 %	Total insects recovered = 23	Eggs added = 171			
B 28	13·9	36 months: attack severe in parts, unattacked wood present	8 (*b*)	2	4	33–36
11	14·3	40 months: attack moderate throughout	1 (*a*)	9	3	38
33	16·1	36 months: attack and decay superficial, slight tunnelling only	1 (*b*) 3 (*c*)	2	—	—
13	16·4	40 months: attack fairly severe but not uniformly distributed	2 (*a*) 3 (*b*)	1	8	36-38
	Av. loss in wt. = 15·2 %	Total insects recovered = 47	Eggs added = 164		Av. minimum life cycle = 37	
C 16	20·5	33 months: attack fairly severe but localized	1 (*b*)	5	1	18–24
10	23·1	33 months: attack severe, reduced to powder at one end	3 (*b*)	—	11	18–24
9	24·3	33 months: attack severe, disintegrated in parts	4 (*b*)	—	7	18–24
(1	18·1	Sample mislaid and not finally cut up but no emergence in 30 months)				
	Av. loss in wt. (3 samples) = 22·6	Total insects recovered = 44	Eggs added = 115		Av. minimum life cycle = 21	
D 34	27·7	33 months: very severely attacked, almost completely disintegrated	—	12	7	18
29	27·7	33 months: extensively powdered except for one strip (? sound heartwood)	1 (*a*)	15	2	18–24
35	30·8	33 months: attack severe but less than in sample 34	1 (*b*)	14	3	15–18
15	30·6	24 months: extensively attacked and powdered	4 (*b*)	10	4	18–24
	Av. loss in wt. = 29·2	Total insects recovered = 73	Eggs added = 167		Av. minimum life cycle = 19	

E	14	35·9	19 months: attack severe in parts, decay not uniform	3 (*a*) 4 (*b*) 3 (*c*) 2 ?	—	1	Not determined but pupa present
	37	35·9	19 months: attack not extensive but powdered in part	2 (*a*) 2 (*b*) 3 (*c*)	7	6	18–19
	6	36·7	19 months: attack moderate: decay not uniform	2 (*a*) 3 (*b*) 1 (*c*)	13	1	15–18
	7	39·3	19 months: attack severe in part: decay not uniform	1 (*a*)	16	2	18–19 (not emerged)
		Av. loss in wt. = 37·0	Total insects recovered = 72	Eggs added = 157	Av. minimum life cycle = 17·5 (emerged)		
F	26	40·5	19 months: attack severe, disintegrated in parts, but some unattacked wood still present	11 (*a*)	3	6	15–18
	32	40·7	19 months: attack severe and extensively powdered	5 (*a*)	9	8	15–18
	39	42·1	19 months: condition as in sample 32	3 (*a*)	15	15	15–18
	30	41·4	19 months: attack very severe, almost completely disintegrated	7 (*a*)	10	8	15–18
		Av. loss in wt. = 41·2	Total insects recovered = 100	Eggs added = 174	Av. minimum life cycle = 16·5		
G	19	45·7	19 months: severely attacked and powdered	2 (*b*)	9	23	15–18
	22	52·0	19 months: very severely attacked and powdered	—	14	10	15–18
	38	47·4	19 months: attack slight in comparison with other samples of group	10 (*a*)	5	1	15–18 (dead: not emerged)
	21	49·2	19 months: attack fairly severe but unattacked wood still present	4 (*a*) 1 (*c*)	—	6	15–18
		Av. loss in wt. = 48·6	Total insects recovered = 85	Eggs added = 169	Av. minimum life cycle = 16·5 (emerged)		
H	1	Controls.	51 months: no trace of attack in any of the samples				
	2	No decay					
	3						
	4						
			Total insects recovered = nil	Eggs added = 156			

* Size: (*a*) $\frac{3}{4}$ to full grown; (*b*) $\frac{1}{2}$ to $\frac{3}{4}$ grown; (*c*) $< \frac{1}{2}$ grown.

place to a varying degree in all samples which had previously been decayed. Furthermore, the extent of decay has obviously a marked effect, determining the rate of larval boring, thereby governing the rate of disintegration of the wood, and also affecting the length of the larval stage and of the life cycle of the insect. For instance, in samples with an average loss in weight through decay of 18 % (group A), the extent of disintegration resulting from insect attack was comparatively slight, and the duration of the life cycle was 55 months, but in severely decayed wood of 73 % loss in weight (group J), the life cycle was completed in less than 12 months and the wood almost completely reduced to powder. Between these extremes, a gradual shortening of the larval stage and decrease in the length of life cycle is apparently associated with an increase in extent of decay.

This relation between the amount of decay in oak sapwood and its suitability for attack has an effect on the insect population in the different samples. The number of larvae which succeeded in starting to bore increased with a rise in the extent of decay in the wood samples. The larval population, therefore, tended to be initially greater in the more highly decayed wood, which, as the results show, was more rapidly disintegrated. Competition for living room more quickly became severe, bringing about the survival to the pupal and adult stages of some individuals, but the death of others (groups G and H). Although the discrepancies between the number of eggs believed to have been added to each group of samples at the beginning of the experiment and the number of insects finally recovered prevent an accurate comparison of groups with regard to the percentage of insects surviving, the indications are, that allowing for the above effect on population, a greater number of larvae completed their development in a shorter time and caused a greater amount of damage as the extent of decay in the wood increased.

(*b*) *Willow decayed by* Polystictus versicolor (*Table* 2).

The results are in general similar to those obtained from the oak-*Phellinus* experiment. The presence of the white rot *Polystictus versicolor* in willow favours its suitability for infestation by the death-watch beetle, and larvae develop more rapidly and cause greater damage in the more highly decayed wood. As in the case of the oak, non-uniformity of distribution of decay in individual samples is the most likely explanation of variation in severity of attack within groups. The occurrence of heartwood, more resistant to decay than sapwood, has also affected the degree of susceptibility of parts of some samples to attack (e.g. A 65 and D 29). It was shown by Fisher (1940) that the heartwood of oak, when decayed, is as suitable for larval development as sapwood, and there is no reason to suppose that this is not true of willow also.

The effect of extent of decay on the insect population in individual samples is again apparent. For instance, in groups F and G with an average loss in weight by decay of 41 and 48 % respectively, a greater proportion of insects succeeded in completing their life cycle in a much shorter time than those in group B, where the extent of decay was only 15 % and the life cycle twice as long. At the same time the number of dead, well-grown larvae found at the final examination of the samples was in general greater in the more highly decayed and extensively powdered samples of groups F and G. This may be explained by the greater suitability of the highly decayed wood for initial and continued larval boring bringing about rapid disintegration of the samples and producing a condition apparently unsuitable for the support of the whole population, with the result that only some individuals

succeeded in completing their development whilst the rest perished. Such a result is the outcome of the limitations of the experiment, and the indications are that with larger samples a higher proportion of the population would have survived and completed the life cycle.

In the oak experiment it was impossible to be certain of the exact number of eggs laid on each sample, but the condition of the willow made it possible to be reasonably sure that the egg counts at the beginning of this experiment were correct. On this assumption the percentage of insects, alive or dead, in each group at the close of the experiment can be compared (Table 3). It should be noted, however, that at the final examination of samples and frass it is unlikely that all larvae of the first few instars were detected, in view of their minute size and shrivelled condition when dead. Table 3 shows that as the extent of decay increased a progressively greater number of larvae succeeded in starting infestation and attained a size which under the conditions of the experiment enabled them to be detected, alive or dead, during examination of the samples. On the other hand, the mortality of young stage larvae not detected increased as the extent of decay decreased. Although the number of larvae reaching the pupal or adult stages does not show a steady increase with increasing extent of decay, it should be recalled that the time factor differed materially in the various

TABLE 3

Loss in wt. by decay %	Eggs added	Insects recovered		Larvae pupated
		Total	% of eggs added	% of eggs added
0·0	97	0	0	0·0
11·1	171	23	13·5	0·0
15·2	164	47	28·7	9·1
22·6	115	42	36·5	16·5
29·2	167	73	43·7	9·6
37·0	157	72	45·8	6·4
41·2	174	100	57·5	21·2
48·6	169	85	50·2	23·7

groups. Thus it took 37 months for 9·1 % of the eggs laid on samples of a loss in weight by decay of 15 % to complete their development, whilst on wood decayed to 40–48 %, 21–24 % of the eggs laid gave rise to adults in only 15–18 months.

B. *Brown rots* (*Table* 4)

Experiments with the cellar fungus *Coniophora cerebella*, which produces a decay of the brown rot type, were not started until the results of the white rot experiments were becoming available. The time required for the preparation of a series of oak samples representing a corresponding range of loss in weight by decay, and the likelihood, as indicated by the results of the white-rot work, of very slow development of the insect in slightly decayed wood, led to the decision to confine experiments with *C. cerebella* to comparatively severely decayed samples. It was hoped in this way to obtain within 2–3 years a general indication of the effect of a brown rot in timber upon its suitability for death-watch beetle attack. In order to facilitate more uniform distribution of decay, smaller samples (6 × 1 × 1 in.) than those used in the white-rot experiments were prepared. Owing to the presence of fissures and the friable condition of the samples after decay, their preparation for egg laying and initial larval boring by means of surface rasping was not necessary. The samples were sorted into three groups of four of approximately equal extent of decay; egg-laying disks were cut from

TABLE 4. *Duration of life cycle in oak sapwood decayed by* Coniophora cerebella *at 22–25° C. and 80–90 % relative humidity*

Group and sample	Loss in wt. by decay %	Duration of attack and final condition of sample	Larvae* recovered: Alive	Dead	Pupated	Minimum duration of life cycle (months)
A 40	33·7	29 months: attack severe; extensively powdered	2 (*b*) 2 (*a*) 4 (*b*) 1 (*c*)	—	4	27–29 (emerged)
6	37·8	29 months: attack moderate; powdered in parts; decay not uniformly distributed	2 (*b*)	—	2	27–29 (emerged)
9†	38·2	29 months: attack very severe; almost completely powdered and disintegrated	10 (*b*)	—	2	27–29 (emerged)
39†	39·1	12 months: attack severe in parts only, remainder unattacked	8 (*b*) 2 (*c*)	— 6 transferred to other samples	—	—
Av. loss in wt. = 37·2		Total insects recovered = 31	Minimum eggs added = 38		Av. minimum life cycle = 28 (emerged)	
B 3†	44·5	22 months: attack slight, unevenly distributed	1 (*b*) 3 (*c*)	—	—	—
13	44·7	14 months: attack severe, extensively powdered in parts	7 (*a*) 3 (*b*)	—	2	< 14 (not emerged)
16	45·3	14 months: attack severe, most of sample powdered	7 (*a*) 7 (*b*) 2 (*c*)	—	—	—
2†	46·4	14 months: attack moderate	1 (*b*)	—	4	< 11 (not emerged)
Av. loss in wt. = 45·2		Total insects recovered = 37	Minimum eggs added = 26		Av. minimum life cycle = < 12 (not emerged)	
C 24	48·7	15 months: attack moderate, but severely powdered at one end	2 (*a*) 8 (*b*) 9 (*c*) 1 ?	—	—	—
21	51·5	12 months: attack moderate, powdered in parts; not uniformly decayed	4 (*a*) 8 (*b*) 10 (*c*)	—	—	—
4†	52·6	12 months: attack severe, extensively powdered	3 (*a*) 3 (*b*)	—	—	—
11†	53·0	12 months: attack very severe, almost completely disintegrated	7 (*b*) 12 (*c*)	— 7 transferred to other samples	2	< 12 (not emerged)
Av. loss in wt. = 51·4		Total insects recovered = 62	Minimum eggs added = 36		Minimum life cycle = < 12 (not emerged)	

* Size: (*a*) ¾ to full grown, (*b*) ½ to ¾ grown, (*c*) < ½ grown. † Samples from which egg-laying disks were cut.

two in each group. Accurate egg counts could not be made, but a minimum of approximately thirty eggs was obtained per group before the disks were replaced on their respective samples and the four bound together for combined infestation by the larvae. The results are summarised in Table 4.

These results confirm those of earlier experiments, in which it was shown that timber decayed by a brown-rot fungus is suitable for infestation by *Xestobium rufovillosum*. In addition, in spite of variations in severity of attack in individual samples resulting from non-uniformity of distribution of decay, there is evidence of more rapid disintegration of the more severely decayed wood, coupled with a decrease in the length of the life cycle of the insect. For instance, in group A with an average loss in weight by decay of 37 %, the average duration of the life cycle was 28 months, whilst in groups B and C averaging 45 and 51 % loss in weight by decay, the minimum length of the life cycle at the time of their final examination was approximately 12 months. Although emergence was not recorded from these groups before cutting up, it can be assumed that if they had remained undisturbed the duration of the life cycle to emergence would not have been increased by more than a few weeks. As in the oak sapwood-*Phellinus* experiments, the absence of precise egg counts precludes a direct comparison between the number of insects recovered per sample and the potential degree of initial infestation. Sufficient evidence is, however, available, from the non-uniformity of distribution of decay to show that in the early larval stages, mortality was high in slightly decayed wood.

The effect of extensive disintegration of the wood upon the development of the larval population is again apparent. For instance, in samples A 9 and C 11, both of which were almost completely reduced to powder by insect populations of 12 and 21 individuals respectively, only two in each sample had reached the pupal stage at the time of final examination. The remaining larvae were half to three-quarters grown, and the condition of the wood was apparently unsuitable for further growth and the completion of their development. There is little doubt that had the samples of decayed wood been sufficiently large, the whole population would have reached the pupal stage with a corresponding increase in the extent of tunnelling and damage caused.

Discussion

The results of the present investigation provide further evidence of the unsuitability of sound timber for initial infestation by first-stage *Xestobium* larvae. When such larvae become established in decayed wood, their rate of boring and growth, which determine the duration of the life cycle of the insect, is dependent upon the extent of decay in the wood in which they are living.

The comparative effect of the decay caused by the different fungi upon the length of the life cycle of the insect can best be shown in graphical form. When the average minimum duration of the life cycle is plotted against the extent of decay, it is clear that a decrease in the life cycle is associated with an increase in the extent of decay, whether of the white or of the brown rot type (Fig. 1). The effect of the presence of fungus is most pronounced at the lower end of the decay scale, and as the fungal attack becomes severe little further effect is apparent. In oak, for instance, the length of the life cycle was not appreciably affected when the extent of decay exceeded 45 %, but in willow the effect of fungal attack reached its maximum more quickly, and changes in the life cycle were only slight in samples decayed

above 27 %. This suggests that slightly decayed willow is more suitable for rapid larval development than oak, in which the extent of decay is also slight. According to Campbell (1930, 1932) and Campbell & Bryant (1940), the effect of *Phellinus cryptarum* and *Polystictus versicolor* upon the chemical composition of wood is essentially similar, but since the density of sound willow is less than that of sound oak, it is conceivable that this difference between these woods might explain the observed differences in the rate of development of the insect in them. Table 5 gives the density of the experimental samples before and after decay, and shows that in both species the initial density was not uniform.

If the duration of the life cycle be plotted against the density of the decayed wood, it is apparent that a decrease in density connotes a shortening of the life cycle. In addition, so far as the white rots are concerned, the rate of development in oak and willow tends to

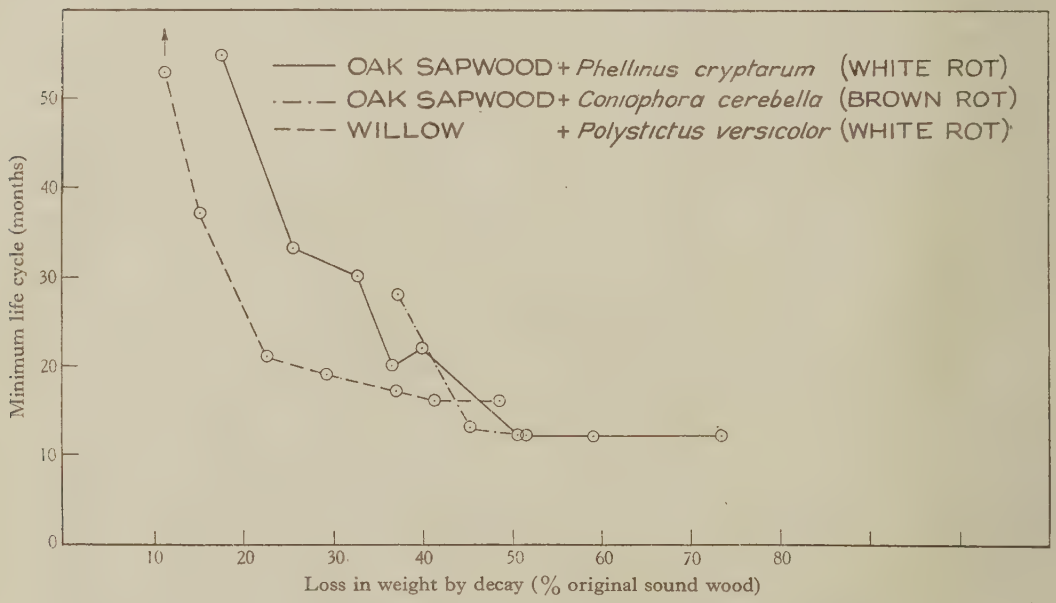

Fig. 1. Relation between extent of decay in oak and willow and duration of life cycle of *Xestobium rufovillosum*.

become the same in both species when the decayed samples are of similar density (Fig. 2). The data obtained from the willow samples for groups B and C, represented on the willow curve by their average, call for comment. Although the initial density and loss in weight by decay in group C were greater than in group B, the final density after decay was similar in both. This suggests that parts of the C samples were more severely decayed than the figures indicate, which would result in an increase in the rate of development of individuals boring in the parts affected, thus explaining the shorter life cycle in group C as compared with group B, although the wood of both was of equal average density.

So far as the effect of the brown rot fungus *Coniophora cerebella* is concerned, the few experimental data available again show that a decrease in the length of the life cycle is associated with a decrease in the density of the decayed samples, the effect being pronounced

TABLE 5. *Density of samples in relation to duration of life cycle*

	White rots								Brown rot			
	Oak + *Phellinus cryptarum* (sapwood)				Willow + *Polystictus versicolor* (heartwood and sapwood)				Oak + *Coniophora cerebella* (sapwood)			
	Density (g./c.c.)		*Loss in wt. by decay	Min. life cycle	Density (g./c.c.)		*Loss in wt. by decay	Min. life cycle	Density (g./c.c.)		*Loss in wt. by decay	Min. life cycle
Group	Before decay	After decay	%	(months)	Before decay	After decay	%	(months)	Before decay	After decay	%	(months)
A	0·493	0·403	17·7	55	0·430	0·384	11·1	>53	0·612	0·383	37·2	28
B	0·485	0·359	25·6	33	0·388	0·329	15·2	37	0·620	0·340	45·2	<13
C	0·497	0·349	32·8	<30	0·425	0·329	22·6	21	0·589	0·286	51·4	<12
D	0·479	0·305	36·5	20	0·405	0·284	29·2	19	—	—	—	—
E	0·486	0·291	40·0	22	0·447	0·282	37·0	17	—	—	—	—
F	Controls				0·429	0·252	41·2	16	—	—	—	—
G	0·539	0·267	50·6	<12	0·400	0·206	48·6	16	—	—	—	—
H	0·520	0·209	61·8	16	Controls				—	—	—	—
J	0·538	0·142	73·3	<12	—	—	—	—	—	—	—	—

* % dry weight of sound wood.

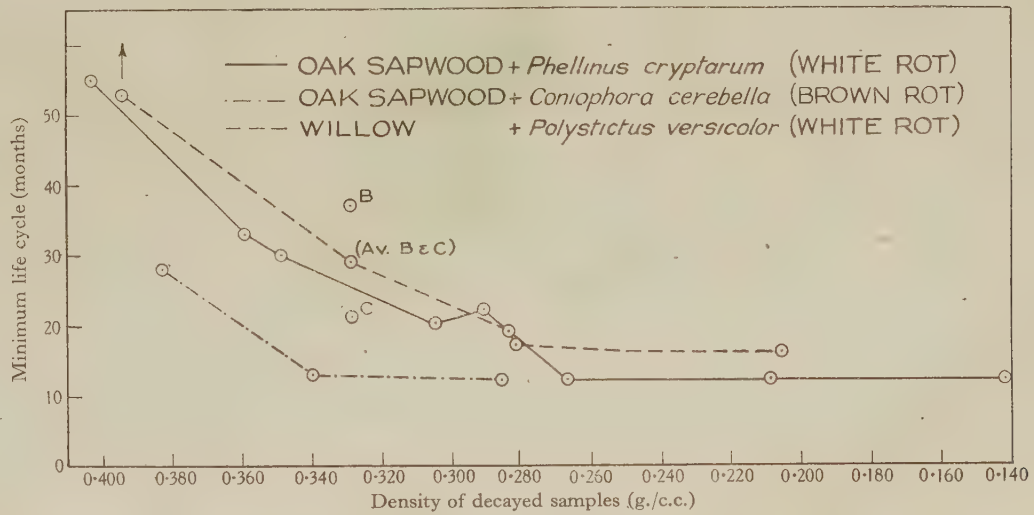

Fig. 2. Relation between density of decayed oak and willow and duration of life cycle of *Xestobium rufovillosum*.

at the lower end of the decay scale. There is, however, also an indication that density is not the only factor involved in determining the apparently greater suitability of oak decayed by this type of fungus for the rapid development of the insect.

It is of interest to compare the above observations and conclusions with the results of the chemical aspect of the investigation (Campbell & Bryant, 1940). In the first instance, these authors determined the effect of *Phellinus cryptarum* upon the chemical composition of sound oak sapwood, and showed that all of the major components were decomposed simultaneously. Comparison of the composition of decayed wood and larval frass led to the conclusion that the food of *Xestobium* larvae consists essentially of wood components and not to any recognizable extent of fungus components, and it is further suggested that the suitability of decayed wood for the development of the insect does not depend on the relative abundance or availability of any food components resulting from fungal activity. Since there is no decline in the dietetic value of oak as it becomes more decayed, it is concluded that the greater suitability of decayed as compared with sound wood for larval development is due to changes in its physical condition and the effect of such changes upon the rate of conservation of energy in the metabolism of the insect.

It has been indicated that there is an apparent close relationship between depreciation in density of decayed wood and the duration of the life cycle of the insect reared in it. Furthermore, the work of Cartwright *et al.* (1936) and of Scheffer (1936) has shown that the rate of decline in strength with progressive decay is greater than the rate of decline in density. On the reasonable assumption that decayed wood offers less resistance to larval boring than sound wood, Campbell & Bryant make use of this relationship between the extent of decay and mechanical strength to bring the results of their chemical investigations into line with the biological observations and reach the conclusion that on an energy metabolism basis, changes in the mechanical strength of wood brought about by decay are the primary cause of variation in the rate of larval development and, thereby, in the duration of the life cycle of *X. rufovillosum*. Although this conclusion is based upon analytical data obtained from experimental work on oak sapwood with *Phellinus cryptarum*, it is supported by the observations on the duration of the life cycle of the insect in willow decayed by the white rot, *Polystictus versicolor*. The difference between the rate of development of *Xestobium* in oak or willow decayed by these fungi and in oak decayed by the brown rot *Coniophora cerebella* may be due to a differential effect upon the mechanical strength of timber attacked by white and brown rot fungi. Unpublished data obtained by the Sections of Mycology and Timber Mechanics of the Forest Products Research Laboratory, Princes Risborough, from experiments upon the influence of *Polystictus versicolor* and *Coniophora cerebella* on the mechanical properties of beech show that for a given loss in density the brown rot caused a larger decrease in strength (compression parallel to the grain) than did the white rot. In these experiments, the effect was examined of losses in weight due to decay less than those used in the present investigation, so that figures are not available for direct comparison with the results of the present work. It can reasonably be concluded, however, that the greater effect of the brown rot upon the mechanical strength of wood and its resistance to larval boring is a factor which may explain the more rapid development of the insect in oak decayed by *Coniophora cerebella* as compared with *Phellinus cryptarum* at the same loss in weight.

These conclusions are of interest in relation to the general problem of the physiology of

nutrition of wood-boring insects. Since Uvarov (1929) pointed out that the food relations of such insects were practically unknown, a considerable advance has been made in knowledge by various workers, but emphasis has been laid by the majority on the physiological relationships between the host insect and intercellular micro-organisms, and to a lesser extent on examination of the enzyme complex present in the gut of wood-borers (Mansour & Mansour-Bek, 1934; Steinhaus, 1940; Parkin, 1940). With a few exceptions, little attention has been given to the significance of the relationship that appears to exist between certain wood-borers and the presence of fungus in timber. Classification of wood-boring insects into ecological groups has been attempted by different workers (Baumberger, 1919; Graham, 1925; Cartwright, 1929; Savely, 1939), but the actual food material utilized has seldom been definitely ascertained. Moreover, so far as the writer is aware, no combined biological and chemical study of the food relations by analysis of food and frass of any species has been undertaken prior to the present work on *Xestobium rufovillosum*. The ecological position of *Xestobium* in the succession of insects which attack the wood of its principal host trees, oak and willow, is difficult to define. The normal habitat of the insect is in the decayed parts of standing trees, but it is seldom found in decayed oak logs. Moreover, in structural timbers it occurs most frequently in decayed wood, but experiment has shown that it can be reared in sound timber, albeit after a prolonged larval period. Previous work by Campbell (1929), Ripper (1930) and Norman (1936), in which a comparison was made of the chemical composition of wood attacked by *X. rufovillosum* with that of the frass of the insect, did not take into consideration the changes brought about in the timber by the previous fungal decay, which has since been shown to precede death-watch beetle damage. The present biological observations have revealed that the mechanical strength of timber, as affected by the extent of fungal decay in it, has a direct bearing upon the rate of larval boring and the duration of life cycle of the beetle, but the implications of the chemical conclusion (Campbell & Bryant, 1940) that the presence of decay has no other major effect upon the timber and its suitability for infestation by the insect are of particular biological interest and call for comment. Sound balsa wood which is of very low density and, therefore, likely to offer less resistance to larval boring as compared with oak and willow, has been shown to be unsuitable for attack (Fisher, 1940). It is conceivable, therefore, that apart from the major physical and chemical changes brought about by fungal attack, micro changes may take place which cannot be detected by existing methods of wood analysis. For instance, the results of chemical examination of infested decayed wood have indicated that fungal mycelium of *Phellinus cryptarum* 'can at most constitute but a small part of the material digested'. No proof is yet available, however, as to whether or not this mycelium might be of direct or indirect value to the insect by bringing about a concentration of pre-existing ferments in the larval gut or by providing a rich source of nitrogen. Campbell & Bryant are of the opinion that the small nitrogen requirements of *Xestobium* are available in sound oak wood and can be utilized directly by the insect. Becker (1938), examining the food relations of the Longhorn beetle *Hylotrupes bajulus*, obtained extremely definite results when the influence of proteins on the feeding of *Hylotrupes* larvae was tested. The weight of larvae fed on pine sapwood to which peptone had been added was on the average 10–15 times as great as that of larvae living in controls. The increase in weight was greater in samples with a higher peptone content, and the rate of development more rapid than in wood with less peptone. He found also that the presence of fungal decay in softwood

timbers resulted in more rapid larval development, as compared with that in sound timber, accompanied by an increase in the amount of damage caused. This result is closely comparable to those obtained in the biological experiments with *Xestobium rufovillosum*. Becker suggests that the effect produced on *Hylotrupes* may be due to concentration of the nitrogenous compounds in the wood.

Furthermore, the observed suitability of decayed timber for rapid larval development of *Xestobium* may also be due in part to the fungus being a possible source of accessory growth substances (Gorcia *et al.*, quoted by Pearse, Patterson, *et al.* 1936), or altering the taste and odour of sound wood, rendering it more palatable to the insect. In general, the relation of *Xestobium* to fungal decay in timber has much in common with a similar association observed in termites, and the following remarks by Hendee (Kofoid *et al.* 1934), on the results of experiments with *Zootermopsis angusticollis* Hagen, have, therefore, a bearing upon the present discussion: 'Better growth and higher viability have been observed among termites of fungus-containing wood than on fungus-free wood. The fungi offer a source of proteins and probably supply vitamins which are essential to the growth and development of termites. Through the secretion of extra-cellular enzymes the fungi may render the wood itself more nutritious. It is not known what effect the fungi may have on harmful extractives in the wood. On fungus-free, sound wood, mortality of termites was even higher during the early part of the experimental period than on the more deficient diet of filter paper, while on sound wood on which a growth of fungus had developed, viability was good. Whether the termites survived because they were better nourished or because the fungi had rendered some toxic factor in the wood harmless, or because of both conditions, is uncertain. It is certain, however, that the differential factor was the presence of the fungus.'

Whilst the work of Campbell & Bryant (1940) has shown that some of these possibilities do not hold for the death-watch beetle, reconsideration by these authors of the analytical data upon which their original conclusions were based, particularly in the light of Becker's experiments, has led to an important modification of their view, as outlined above, of the nutritional significance of the presence of decay in timber for the larva of *Xestobium*. The re-stated chemical case will be published elsewhere, but the authors have kindly suggested that a summary of their main conclusions be included in the present discussion. Depreciation in the mechanical strength of wood resulting in decreasing resistance to larval boring has been shown from the biological observations to be especially advantageous to the young larva, enabling it to start boring, and once established to continue at a greater rate than in sound wood.

This loss in mechanical strength through fungal attack is brought about by the decomposition of wood components, of which a large proportion is dissipated as carbon dioxide and water, in the metabolism of the fungus. As the fungus does not remove nitrogen from wood at anything approaching the rate at which it removes the major components, the net nitrogen content of decayed wood tends to increase as the extent of decay increases. In their original work Campbell & Bryant, expressing the amount of wood digested by a group of larvae as a percentage by weight of the wood disintegrated during boring, obtained an 'abstraction coefficient', which they used as a direct measure of the amount of energy abstracted by the larvae per unit weight of wood disintegrated.

It has now been found that despite the decline in the abstraction coefficient, which was shown to accompany an increase, over a wide range, in extent of decay, an adequate balance

of nitrogen is maintained in the wood actually digested. Since decay reduces resistance to boring, the larva is enabled to conserve nitrogen at a greater rate and, therefore, to complete its development more quickly in decayed than in less decayed, or sound wood.

Such an interpretation of the results of the chemical work is in closer agreement with the biological observations than the original chemical conclusions, since it now appears that in the relationship which exists between type and extent of fungal decay in timber, and rate of development of *Xestobium*, the nutritional value of the decayed wood in relation to its nitrogen content and distribution, combined with the effect of decay on its physical condition are of prime importance. Other factors may also operate, but it can be concluded that under favourable conditions of temperature and humidity, the degree of susceptibility of a suitable timber species to attack by the death-watch beetle is determined primarily by the extent of fungal decay in it.

Summary

The biological aspect of the effect of fungi producing decay of the white and brown rot types upon the rate of development of *Xestobium* larvae and the duration of life cycle of the insect has been studied. An account of the chemical work involved has been published separately.

The minimum duration of the life cycle at 22–25° C. and 80–90 % relative humidity (equivalent moisture content of wood 18–20 %) has been determined in a series of samples representative of a range of different degrees of decay, loss in oven-dry weight due to fungal attack, expressed as a percentage of the original dry weight of the sound wood, being taken as a measure of the extent of decay. Oak sapwood decayed by *Phellinus cryptarum* (Karst.) (a white rot) and *Coniophora cerebella* (Pers.) (a brown rot), and willow decayed by *Polystictus versicolor* (Linn.) Fr. (a white rot) were used.

It is shown that sound timber is unsuitable for infestation by first-stage larvae. In wood decayed by fungi of the brown or white rot types, the rate of boring and development of the larvae increases as the extent of decay increases.

The rate of larval development and the duration of the life cycle are also shown to be affected by changes in density and mechanical strength of timber resulting from fungal attack.

These findings are discussed in relation to the published results of the chemical aspect of the investigation, which suggest that depreciation in mechanical strength is the major effect of fungal decay responsible for the decrease in the duration of the life cycle associated with an increase in the extent of decay. The significance of this conclusion is examined in relation to the physiology of nutrition of wood-boring insects, and it is concluded from the biological results of the present investigation that sufficient evidence is not available to show that changes in the nutritional value of the wood which might affect the rate of development of the insect do not occur as a result of fungal decay.

Re-consideration by the wood chemists of the chemical data already published has revealed that the nitrogen metabolism of the insect is affected by the presence of fungal decay in timber. The nutritional value of decayed wood in relation to its nitrogen content and distribution, together with the depreciation in mechanical strength which results from fungal attack, is now concluded to be of prime importance in determining the degree of suitability of timber for infestation by the death-watch beetle and the rate of development of the insect in it.

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SHEEP BLOW-FLY INVESTIGATIONS

IX. ON SOME PHYSICAL ASPECTS OF SHEEP DIPPING

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(With 6 Text-figures)

Although a great deal of research has been carried out on the relationship between the physical properties of horticultural spray fluids and their insecticidal and fungicidal value, little information is available concerning this aspect of sheep dips. The insecticides present in dips serve two purposes—killing insects already living in the fleece, and protecting the sheep against future infestation by pests such as blow-fly maggots. The direct killing effect requires intimate contact between the dipping fluid and the insect, but the protective action depends on the deposit of poison left in the fleece after the dip has dried. The physical properties of an aqueous spray or dipping fluid can be profoundly influenced by the addition of auxiliary materials commonly known as spreaders or wetting agents. Soap is the best known example, but there are now available numerous synthetic products which exert a similar effect; many of these were developed in the first instance for the scouring of wool. The effect of spreaders is not limited, however, to increasing the ability of the liquid to spread over a plane surface; they also improve the wetting, penetrating and detergent properties, and it is these which may be of importance in dipping fluids.

It is usually recommended that sheep should be dipped for 1 min., but, in practice, it is quite common for the period of immersion to be less than 20 sec. The ideal dip for killing external parasites should, therefore, penetrate the fleece very rapidly and wet the wool and any insects present. With regard to the protective action of dips, the problem is rather different; rapid penetration is clearly desirable, but it is also important that a satisfactory deposit of the insecticide should remain in the wool. After a sheep has emerged from the dipping bath and the excess liquid has run off, there still remains a large amount of liquid which is lodged in the fleece and can be squeezed out by pressing the wool. It seems likely that, if the penetrating properties of the dip are increased, the fluid will run out of the fleece more readily, a smaller amount of the protective material remaining; on the other hand, a more even impregnation of the fleece and skin should result. An investigation was, therefore, made on the effect of wetting agents on the retention and distribution of dip in the fleece and on the protection afforded against sheep maggots. A preliminary account of some of these experiments was given by Hobson (1939). Insecticides may occur in sheep-dips as water soluble substances, insoluble powders or oil emulsions. The present investigations deal mainly with water-soluble arsenic; this is the chief constituent of the arsenic-sulphur preparations which are the dips commonly used for protecting sheep against blow-fly attack.

Experimental

Methods

These investigations were started by comparing sheep dipped in a proprietary arsenic-sulphur powder dip with other animals dipped in the same bath after the addition of the experimental wetting agent. Usually, three sheep were used in each test, the period of immersion being 1 min.; an experiment will be described later on the effect of time of immersion on arsenic retention by the fleece. Samples of wool were collected from the sheep at intervals for analysis; each sample was composed of four subsamples taken in a standard way along the middle of the back. The protective value of the dip was assessed by a modified form of the method proposed by MacLeod (1937); this procedure was described by Hobson (1940), and consists essentially of placing eggs of *Lucilia sericata*, the sheep maggot-fly, on the skin under a plug of damp cotton-wool. With regard to the sheep (Welsh) used in these experiments, the expression 'lambs' refers to lambs aged 3–6 months; 'yearling' will be used to designate sheep born in the previous season and aged 9–15 months.

Water-soluble arsenic was determined as follows: the wool, 0·5–1 g., was extracted with water and the organic matter in the extract destroyed by evaporation with sulphuric and nitric acids; the arsenic was then reduced with hydriodic acid to arsenite and titrated with *N*/50 iodine in neutral solution. Acid-soluble arsenic was determined in a similar way in extracts made with dilute sulphuric acid. The results were calculated as percentages of the weight of the crude wool after air drying. Laboratory methods were devised for measuring the retention of liquid by wool and the leaching of arsenic from dipped wool; these will be described with the experimental results. In the investigations on the effect of wool constituents on the dipping fluid, surface tension was determined by means of Traube's stalagmometer.

Table 1. *Results of myiasis experiments*

Days after dipping	Dip: Arsenic		Dip: Arsenic + Agral 2	
14	−	−	+	−
20	−	−	+	−
30	−	−	+	+
35	+	−		

\+ Maggots grew and produced wound. − Maggots died.

Table 2. *Arsenic content of wool from base of fleece*

Lamb no.	Dip	% water-soluble As_2O_3			% acid-soluble As_2O_3		
1	Arsenic dip alone	0·51	0·60	0·59	0·29	0·19	0·10
2	,,	0·36	0·36	0·47	0·16	0·28	0·23
3	Arsenic dip + Agral 2	0·39	0·30	0·28	0·08	0·09	0·09
4	,,	0·25	0·28	0·16	0·10	0·07	0·08
Days between dipping and sampling		4	11	21	4	11	21

Table 3. *Content of water-soluble arsenic in basal wool 2 days after dipping*

Lamb no.	Dip	% As_2O_3
5	Arsenic dip alone	0·45, 0·46
6	,,	0·56, 0·60
7	Arsenic dip + Sulphonated Lorol	0·33, 0·35
8	,,	0·34, 0·34

Dipping tests with added wetting agents

Experiments were carried out with two proprietary wetting agents added to an arsenic-sulphur powder dip: Agral 2, 8 oz./100 gal. and Sulphonated Lorol, 12 oz./100 gal. The results of the myiasis tests (Table 1) show that the addition of Agral 2 seriously impaired the protective action of the arsenic dip. With regard to the analysis of the wool from these yearlings (Table 2), the results for soluble arsenic are somewhat erratic, but the wetting agent appears to decrease the amount retained in the fleece and to cause a loss of arsenic subsequent to dipping. The results for insoluble arsenic show the effect of the wetting agent more clearly; the figures are lower and far more consistent with the dip containing Agral 2. In the experiment with Sulphonated Lorol, the analyses (Table 3) show that the addition of this wetting agent also decreased the retention of soluble arsenic in the fleece.

In both these experiments, heavy showers of rain fell during the night following the dipping, before the sheep were dry. It seems likely that a wetting agent may render the fleece more permeable to rain; consequently, the decrease in arsenic content, observed when wetting agents were added to the dip, may have been due to the leaching action of rain. However, laboratory experiments on the retention of water by wool suggest that wetting agents also decrease the initial amount of dip retained by the fleece.

Effect of wetting agents on the retention of water by wool

A laboratory method was worked out for studying this problem, the amounts of liquid retained by a sample of wool being determined when the wool was dipped, first in water, then in experimental solutions. This method proved useful in obtaining preliminary information about various wetting agents at different concentrations. Pieces of skin, about 0·5 in. square, were cut out of a fleece as required. The skin was attached to a safety pin, immersed in water with the wool downwards, and left to drain; any moisture on top of the skin was removed with filter paper. After the wool had drained for 10 min. the amount of liquid retained was found by weighing. Provided the wool was not disarranged by violent agitation, consistent values were obtained once the wool became thoroughly wet; the wool was then dipped in experimental solutions, the results being expressed as percentages of the

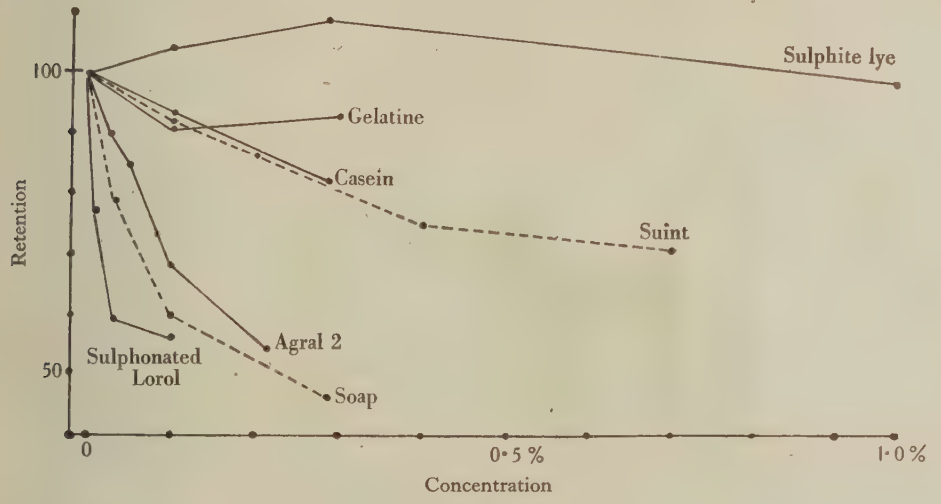

Fig. 1. Effect of various added materials on the retention of water by wool.

value obtained with pure water. The soap solution was prepared by dissolving 10 ml. of oleic acid in 990 ml. of water containing 3 g. of caustic soda; the concentration of soap in this solution was taken as 20 %. The sample of sulphite lye was of the soda base type, its density being 60° Tw. The casein was dissolved in water with the minimum amount of caustic soda. The suint solution was prepared by extracting crude wool with water and filtering.

When the fleece sample, after being dipped in water, was transferred to a solution of a wetting agent, the wool lengthened considerably and the liquid ran out quickly in small drops. Fig. 1 shows the results of the retention determinations. The highly surface-active materials, soap and the proprietary wetting agents, produced a marked decrease in water retention at low concentrations. Gelatine and casein, which have been used as spray supplements on account of their adhesive properties, also decreased retention. Sulphite lye was the only substance found to increase the amount of liquid retained by wool. The most interesting result is that obtained with suint, the soluble material present in sheep's wool, which was found to decrease the retention of liquid. Suint, together with urine and dung must accumulate in the dipping bath when sheep are dipped; experiments were, therefore, carried out to determine whether the retention of arsenic by the fleece and the protective properties of the dip are affected after a large number of sheep have passed through the dipping bath. Before these experiments are described, the properties of suint will be briefly reviewed.

Properties of suint

Suint is the water-soluble fraction of wool yolk; it is dark coloured and extremely hygroscopic when dried. Freney (1940) showed that soaps form a large proportion of the organic matter, the fatty acid content of suint varying from 11 to 20 %. The fatty acids in suint are probably not identical with those present in natural oils and fats. It has long been known that suint has good detergent properties and these are utilized in the Duhamel continuous process for scouring wool. Stott & Mengi (1934) examined the physical properties of suint and concluded that its surface activity is due to the soaps present; they found that suint depressed the interfacial tension of water against benzene, the decrease with strong solutions being greater than the maximum decrease with potassium oleate. In the present investigation, surface tension was used for measuring the surface activity of suint and dipping fluids; Fig. 2 shows the values obtained with a freshly prepared extract of suint.

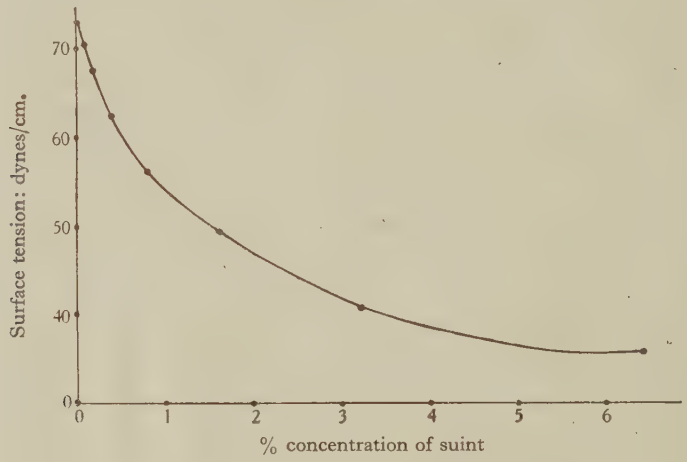

Fig. 2. Surface tension of aqueous solutions of suint.

TABLE 4. *Effect of suint on dipping fluid*

Sample	Period of immersion sec.	Surface tension dynes/cm.	% suint
Initial dip		73·0	0
Drainings from 1st sheep	60	65·9	0·19
Drainings from 2nd sheep	60	65·3	0·23
Dip after 3rd sheep	—	68·3	0·05
Drainings from 4th sheep	45	63·4	0·03
Drainings from 5th sheep	30	58·1	0·51
Dip after 5th sheep	—	65·5	0·17

Effect of suint on sheep dips

A preliminary experiment was carried out with five yearlings dipped in a bath containing 100 gal. of arsenic-sulphur powder dip. Samples were taken of the dipping fluid and of the dip running out of the fleece about 1 min. after the sheep had emerged from the dip. Table 4 shows the values for surface tension and suint content; this was estimated by determining the soluble matter and subtracting the amount supplied by the dip.

The soluble compounds in wool are only partially removed during dipping and solution probably continues after the sheep has left the bath. Thus, with the 1st yearling to be dipped, the liquid draining from the fleece contained an appreciable quantity of suint, the value for surface tension being noticeably lower than that of the fresh dip. The evidence also suggests that the suint content of the drainings tends to increase with a shorter period of immersion. With regard to the dipping fluid, the passage of

only five sheep through 100 gal. of dip decreased the surface tension from 73·0 to 65·5 dynes/cm. A further experiment was, therefore, carried out with a large number of sheep.

264 Welsh yearlings were dipped on 12 April 1939; the bath was filled with 240 gal. of arsenic-sulphur powder dip, and was twice replenished with 40 gal. of dip; the residue after dipping amounted to 145 gal. These volumes are only approximate. The original dip was made up under strength, but the bath was replenished with full strength dip; consequently, the arsenic content was slightly higher at the end. The first and last three sheep through the bath were kept under observation. Myiasis experiments were carried out with these sheep and analyses were made of wool samples and of the dipping fluid. The wool samples were collected at intervals after dipping, each sample being divided into three fractions, the outer stained tip, a basal fraction about 1·5 cm. long, and the remaining middle fraction.

Table 5 shows that the sheep dipped last proved more susceptible to artificial maggot infestation than those dipped first; the question of the effect of suint on the protective action of fly dips will be discussed later. Table 6 shows that a considerable amount of soluble matter had accumulated in the bath after 264 sheep had been dipped; also, there was a striking decrease in the surface tension of the

TABLE 5. *Results of myiasis tests*

Days after dipping	First 3 sheep dipped	Last 3 sheep dipped	Controls
12	− − −	+ ± −	+ + + −
15	−	+	+ +
17	− −	+ −	+ −
19	+ −	+ +	+ +
21	+ −		
26	+ −	+ +	+ −
29	+ −		+ +

\+ Maggots grew and produced wound. ± Maggots alive, no wound. − Maggots died.

TABLE 6. *Effect of the presence of suint in the dip*

	Initial dip	Final dip
% soluble As_2O_3	0·118	0·125
% soluble matter	0·19	2·66
Surface tension: dynes/cm.	73·0	43·3

% soluble As_2O_3 in wool 6 days after dipping	First 3 sheep dipped	Last 3 sheep dipped
Base	0·51, 0·44, 0·58	0·12, 0·23, 0·25
Middle	0·34, 0·23, 0·22	0·14, 0·13, 0·15
Tip	0·44, 0·44, 0·42	0·11, 0·14, 0·12

liquid. The sheep used were Welsh yearlings in almost full fleece, but they were small and in poor condition after a hard winter. Higher concentrations of suint probably occur at larger dippings, especially of lowland sheep in good condition. Table 6 also shows that there was a pronounced difference between the sheep dipped at the beginning and at the end, the arsenic content of the fleece being considerably lower in the sheep dipped last. This effect must have been due to the extraneous material introduced by the sheep, since the dip did not become weaker. Although the laboratory experiments (Fig. 1) had suggested that contamination with suint would lower the retention of dip by the fleece, the decrease found was surprisingly large, about 50 %. This may have been due to the high content of soluble matter in the final dip, over 2 %, and to the presence of urine. In addition, some arsenic must have been washed out of the wool by rain before the first series of samples were collected; the analyses of the later samples showed that the loss of arsenic from the fleece was more rapid in the group of sheep dipped last.

The results of these analyses are given in Table 7. Figs. 3 and 5 show, respectively, the distribution of arsenic along the staple, and the relative loss of arsenic from different fractions of the fleece. These diagrams are constructed from the average values of samples from three sheep. For comparison, the results of a further experiment are given in Fig. 4. In this case, the sheep (No. 1, Table 4) was dipped

in fresh dip, and duplicate sets of wool samples were collected at intervals, the fleece being divided into four fractions. All these wool samples were taken from the back.

TABLE 7. *Arsenic content of fleece at intervals after dipping*

Days after dipping ...	% soluble arsenic					
	26			50		
	Base	Middle	Tip	Base	Middle	Tip
First 3 sheep dipped	0·41	0·31	0·32	0·17	0·30	0·26
	0·25	0·26	0·27	0·12	0·27	0·22
	0·32	0·29	0·29	0·14	0·33	0·25
Last 3 sheep dipped	0·11	0·06	0·05			
	0·08	0·05	0·05	0·029	0·081	0·053
	0·09	0·06	0·06	(3 samples mixed in equal proportions)		

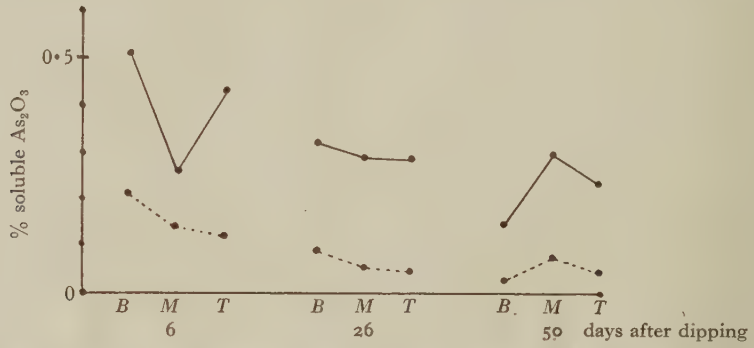

Fig. 3. Distribution of soluble arsenic along the staple following dipping. Full line, first three sheep dipped; broken line, last three sheep dipped. B=basal, M=middle, T=top, third of fleece.

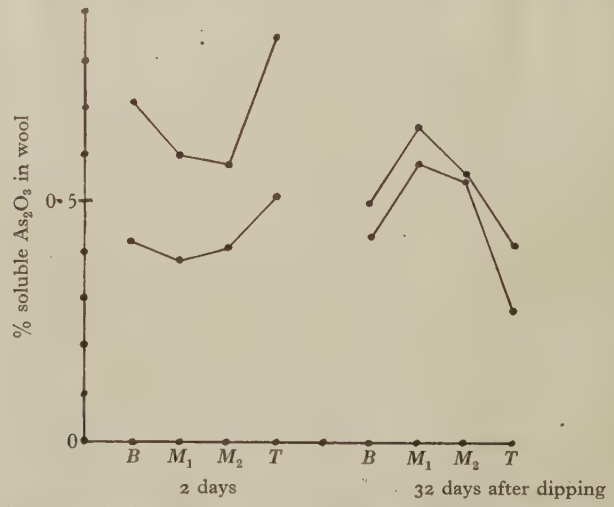

Fig. 4. Distribution of soluble arsenic along the staple following dipping. B=basal fraction; M_1, M_2=middle fractions; T=top fraction. Lines join results of duplicate sets of samples from one sheep.

The initial distribution of soluble arsenic along the staple was similar in all the sheep immersed in fresh dip, the middle fraction containing the least arsenic. The distribution is later completely reversed as the arsenic content decreases at the base and tip, remaining constant in the middle. This indicates

that the leaching of soluble arsenic by rain was confined to the outer part of the fleece, as the apparent loss of arsenic at the base can be explained by the growth of new wool. From the results shown in Fig. 5, it was calculated that the wool must have grown at least 1 cm. in 44 days to account for the loss. No observations on wool growth were made on these sheep in 1939, but wool measurements were carried out in 1940 with similar sheep at the same time of year; on shaved areas of skin, the wool grew 0·9 and 1·3 cm. on two sheep in 31 days.

When the dip had become contaminated with suint, the initial distribution along the staple was quite different, the arsenic content decreasing from base to tip. It will be noted that these samples were not collected until 6 days after dipping, and a considerable amount of rain fell during the interval; however, Fig. 5 suggests that this should not affect the distribution along the staple. With the sheep dipped last, in contrast to those dipped first, all the fractions lost arsenic at about the same rate during the first period, which suggests that rain was penetrating farther than the outer part of the fleece; in the second period, the loss was confined to the tip and base, as in the case of sheep dipped first. A

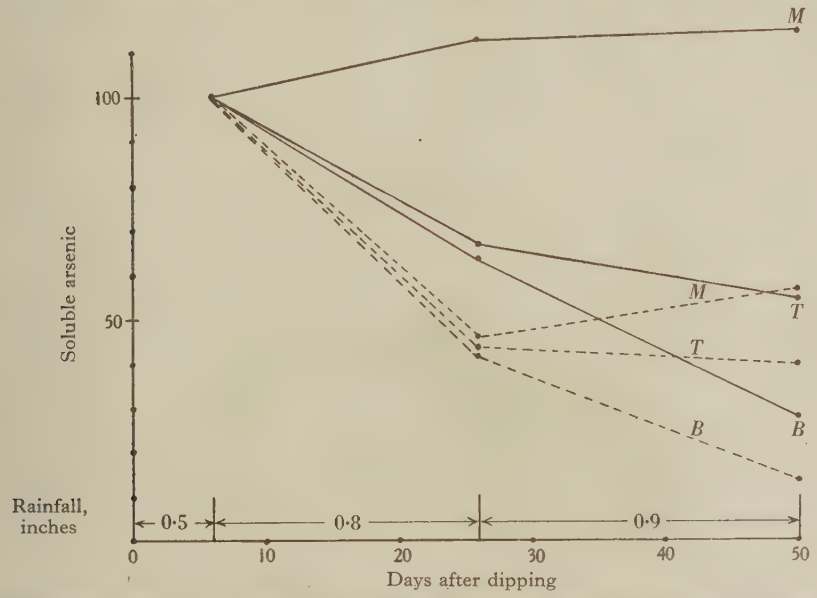

Fig. 5. Relative loss of soluble arsenic from different fractions of fleece after dipping. Full line, first three sheep; broken line, last three sheep dipped. *B*, *M*, *T*, basal, middle, top fractions respectively.

possible explanation is that, with the contaminated dip, the extra suint in the fleece rendered it permeable to rain; the amount of suint would be reduced by leaching during the first period, the fleece thus becoming impermeable in the second period. Unfortunately, the suint content of the samples was not examined, but from analyses of wool from sheep which had been double dipped (Hobson, 1936), it seems probable that a single dipping removes at least half of the suint present in the fleece. The writer has observed, when carrying out suint analyses, that wool rich in suint is more easily wetted with water than wool poor in suint, but a difficulty about this explanation is the fact that a considerable amount of suint remains in the fleece of sheep dipped at the beginning; the difference between suint content in the two groups would not be very great even with the dip at the end containing about 2·5 % suint. However, there is a wide variation in the amount of suint present in different parts of the fleece; thus, in samples taken from a ewe in full fleece, the suint content was over 20 % at the base of the staple on the chest, and 1·5 % at the tip on the back (Hobson, 1936). Assuming that the sheep used in the dipping experiment contained 1·5 % of suint in the outer fraction of the wool on the back, and that immersion removed half of the suint present, then in the sheep dipped first the amount would fall to 0·75 %, but, in the sheep dipped last, the suint content would rise to 3·25 %, this value being calculated on the arsenic analyses. The difference would, therefore, be considerable in the outer fraction of fleece on the back, which is the part exposed to rain.

Experiments on the extraction of arsenic from wool

Laboratory tests were carried out to determine whether the presence of suint accelerates the removal of arsenic when wool is immersed in water. The wool samples, with the skin attached, were taken from a skin, dipped in the experimental solution and conditioned for a week at 50 % relative humidity and 35° C.; they were then immersed in water at 35° C. for 1 min. under standard conditions, and left to drain, the amount of arsenic in the extract and in the wool being determined. Two dips were used, together with various added materials: (*a*) the soluble fraction of a proprietary arsenic-sulphur dip; (*b*) a suspension of zinc arsenite. The results are given in Table 8.

The soluble arsenic was not completely removed by immersion in water, but more arsenic was dissolved out if the dipping solution contained suint. This effect of suint was not prevented by the addition of various stickers. A temperature of 35° C. was chosen, so as to imitate conditions at the base of the fleece; substances such as glue and dextrin probably lose their adhesive properties at this temperature. Zinc arsenite, an insoluble powder, adhered well to the wool, but addition of suint to the dipping fluid considerably decreased its resistance to treatment with water. Sulphite lye has, like suint, good detergent properties and produced a similar result, whereas Agral 2 had little effect. Fajans & Martin (1937) suggested that the tenacity of insoluble deposits is reduced by the incorporation of detergents. Suint, in addition to being a detergent, is also extremely hygroscopic. When dried at 50 % relative humidity at 35° C., approximately the conditions at the base of the fleece, suint forms a viscous liquid having about the consistency of treacle. Presumably, the fibres become coated with a thin film of this liquid when the fleece dries after dipping.

TABLE 8. *Effect of auxiliary materials on the extraction of arsenic from wool*

	Dip used	% of arsenic removed from dipped wool by subsequent treatment with water
1	Soluble arsenic	40, 53
2	Soluble arsenic + 2 % suint	63, 75
3	As_2O_3 + 0·2 % dextrin	67, 91
4	As_2O_3 + 0·2 % gelatine	67, 71
5	As_2O_3 + 0·7 % oil emulsion	75, 75
6	Zinc arsenite	7, 10, 11
7	Zinc arsenite + 2% suint	57, 59
8	Zinc arsenite + 0·05 % Agral 2	20, 20
9	Zinc arsenite + 0·5 % sulphite lye	36, 46
10	Zinc arsenite + 1·0 % sulphite lye	38, 43

TABLE 9. *Distribution of arsenic over the body shortly after dipping*

Sheep	Date of dipping	% soluble As_2O_3 in wool from base of fleece: Back	Rump	Middle of ribs	Chest
1 Yearling	Mar.	0·64, 0·69	0·29, 0·41	0·49	0·40
2 Lamb	Aug.	0·30	0·22	0·18	—
3 Lamb	Aug.	0·19	0·10	0·11	—
4 Lamb	Aug.	0·36	0·20	0·18	—

Distribution of arsenic over the body

This question was investigated by collecting wool samples from various parts of the body shortly after dipping. The lambs were dipped in August when the wool was short, the yearling in spring when the wool was long. The results of the analyses, given in Table 9, show that the arsenic content of the wool is lower on the sides of the body than on the back. This may be due to the fact that the dip drains more readily from the sides; in addition, the wool is shorter. Since the tail region is a common site of infestation by maggots and the first strikes after dipping usually occur in this region, the arsenic content of the wool on the rump is of special interest. The arsenic content of the fleece was found to be lower with the lambs than with sheep having longer wool.

Effect of time of immersion

On the majority of farms, at summer dippings, the sheep are in the bath for less than 30 sec. In order to investigate the effect of the time of immersion, two groups of lambs were dipped for 15 and 30 sec. respectively; wool samples were collected the following day and analysed for arsenic. The results in Table 10 show that there was no appreciable difference between the two groups.

As heavy rain fell in the week following this experiment, further groups of samples were collected 1 and 3 weeks after dipping. Fig. 6 shows the mean figures obtained from samples from three lambs; the wool was taken from the back and divided into two fractions.

Both fractions of the fleece lost soluble arsenic during the first week after dipping, presumably due to leaching by rain. However, water could not have penetrated far into the fleece since the decrease was much greater in the outer part than at the base, the relative losses being respectively 75 and 35 %.

TABLE 10. *Effect of time of immersion on the arsenic content of the fleece*

% As_2O_3 in wool from base of fleece on back 1 day after dipping.

	Soluble	Insoluble		Soluble	Insoluble
3 lambs dipped 30 sec.	0·30	0·048	3 lambs dipped 15 sec.	0·24	0·032
	0·19	0·014		0·31	0·018
	0·36	0·025		0·20	0·036
Mean value	0·28	0·032		0·25	0·029

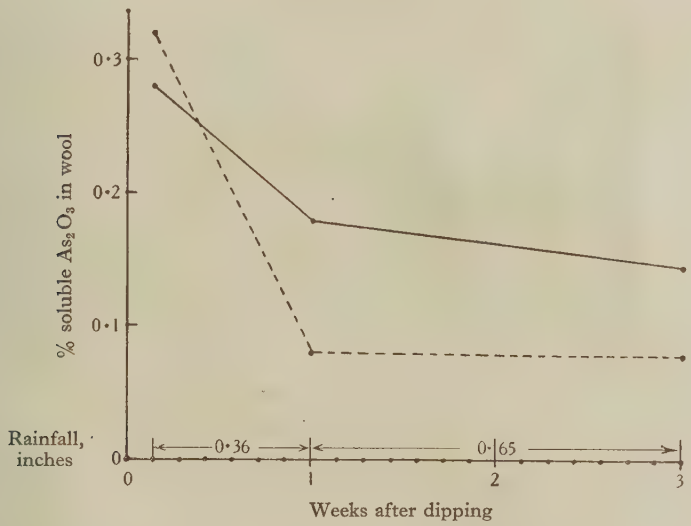

Fig. 6. Arsenic content of wool of lambs following dipping.
Full line, inner half of fleece; broken line, outer half.

In the second period, there was practically no loss of soluble arsenic from the fleece, although a considerable amount of rain fell. Possibly, the compounds which are more readily soluble, or the more accessible parts of the deposit, were removed during the first period. An alternative explanation is that some change occurs in the wool yolk a few days after dipping, which protects the soluble fraction against leaching; the suint may become more intimately associated with the grease, or the film of dip on the outside of the fibres may be covered with a fresh secretion of wool grease. In the dipping experiments carried out with yearlings in March and April, the leaching action of rain seemed to be confined to the outer fraction of the fleece (p. 265). The different result obtained with lambs in August was presumably due to the nature of the wool; these animals were small, and the length of the fleece was about 4–5 cm. The fleece of the yearlings was longer, about 6–8 cm., and considerably denser. These measurements apply to the fleece *in situ*, and not to the fibres when extended.

Discussion

The sheep's fleece is well adapted for the important function of keeping out rain, as the wool fibres are coated with a film of grease secreted by the sebaceous glands. If a sheep were dipped in a liquid capable of wetting the fleece perfectly, the excess liquid would drain away after immersion, leaving a continuous film over the wool. However, water has poor wetting properties for the greasy surface of the wool fibres; after dipping, the liquid will be present as surface films and as small droplets adhering to the fibres, also as larger drops entangled in the fleece. The latter will be referred to as free liquid, although it is unlikely that the two forms are sharply differentiated. The free liquid will tend to be dislodged by the sheep shaking itself and the amount retained will depend on the type of fleece. This is shown by the results of the wool analyses. With yearlings in long wool, the soluble arsenic content of the fleece after dipping varied from 0·36 to 0·71 % (basal wool), the average being 0·47 %; with young lambs, the values ranged from 0·19 to 0·36 %, with an average of 0·25 %. As the dip contained 0·12–0·13 % of soluble arsenic, the wool, therefore, took out four and two times its weight of liquid respectively. Evidently, the shorter and more open fleece of the younger lambs retained less free liquid than the longer and more compact fleece of the older animals.

The dipping experiments have shown that the fleece retains less dip if a wetting agent is present. The increase in penetrating powers is likely to cause the free liquid to drain from the fleece to a greater extent. Also, the improved wetting properties may result in a decreased retention of the small droplets adhering to the fibres, as these will tend to spread out in a thin film. A reduction in the amount of dip left in the fleece does not necessarily imply a decrease in the protection afforded against sheep maggots. It is the poison on, or close to, the skin which is effective against this pest, and penetration of the dip to the base of the fleece is, therefore, essential. The present experiments were carried out with Welsh sheep whose fleece is fairly open; good penetrating properties may be more important for close-woolled sheep, such as Down breeds.

The original aim of the present work was to find out whether it is desirable to add a wetting agent to fly dips, but the investigation took rather a different course when the effect of suint was discovered. This suggested that it is unnecessary to add a wetting agent to powder dips as the soap present in the fleece is dissolved out during dipping, and accumulates in the bath. Little is known about the effect of various surface-active materials on the wetting and penetrating powers of sheep dips, and evaluation of these properties is difficult. Measurement of surface tension does not assess the efficiency of wetting agents accurately, but it is probably safe to assume that the wetting and penetrating properties of the dip will be considerably improved when the surface tension falls to the extent observed in these experiments. The chief value of an added wetting agent may lie in the preparation of the dip. The mixing of an arsenic-sulphur powder is a tedious operation and the sheep may be injured if the dip is prepared carelessly and some of the powder floats on the surface. By the addition of suitable wetting agents, the dip may be supplied as a powder, which can be poured into water without pasting. However, the results obtained with a proprietary wetting agent, Agral 2, and with suint, suggest that these substances decrease the protective properties of fly dips.

It has long been realized by farmers that the dipping fluid becomes less effective for the

protection of sheep against maggot flies after a large number of sheep have been through the bath. This has usually been ascribed to contamination of the dip with dung and urine, materials which attract the fly; although undesirable, soiling of the wool with excretions should not matter, *provided* a satisfactory deposit of insecticide is obtained in the fleece. The present results suggest that contamination with suint may be more serious since this changes the physical properties of the liquid and results in a smaller and less stable deposit of poison in the wool. If the hypothesis is correct that suint tends to make the fleece permeable to rain (p. 267), the presence of this material in the dip will exert a further harmful effect on the sheep dipped last. In wet weather, the extra suint will cause a higher humidity in the fleece, and this will render the sheep more susceptible to maggots. This factor does not, however, enter into the artificial myiasis experiments in which the humidity is controlled by means of a moist cotton wool plug. An attempt was made to find some means of preventing the deleterious effects of suint, but no practical method was discovered. A preliminary washing might be possible on some farms, but the sheep would have to be dry before the actual dipping. The chief recommendation is that the dip be changed at fairly frequent intervals.

The effect of suint on the physical properties of dipping fluids has not apparently been investigated before, although Hambrock *et al.* (1934) suggested that the soap in suint might reduce the surface tension of the dip and increase its wetting properties. This is confirmed by the present observations which have shown that the surface tension of the dip falls rapidly as the sheep pass through the bath. Apart from the question of blow-fly strike, the presence of suint in the bath liquor may affect the behaviour of sheep dips in various ways. The direct insecticidal action will tend to increase, but the protection afforded against various pests may be diminished owing to the decrease in retention. The change in the physical properties of the liquid may also influence the effect of the dip on the skin and wool. The effect of suint should be taken into account when sheep dips are being tested; the first few sheep through the bath will be abnormal and more typical results will be obtained when a hundred or so sheep have been through the bath.

The penetration of the fleece by rain and the leaching of soluble arsenic from the fleece have recently been the subject of controversy. Investigations on the humidity conditions in the fleece (Davies & Hobson, 1935; MacLeod, 1940) indicate that rain seldom penetrates to the base of the wool in British breeds of sheep in this country. Lennox (1938) stated that in Australia palpable free moisture occurs at the base of the fleece in Merinos following heavy rain. The different results obtained in Australia may be due to the breed of sheep. MacLeod observed that penetration is greater with close-woolled fleeces than with open-woolled fleeces; the wool of Merinos is much closer than that of any British breed. With regard to the leaching of arsenic from the fleece, Moore (1937) suggested that water-soluble substances are readily washed off by rain; he was unable to detect soluble arsenic in the *outside* of the fleece 15 days after dipping, these observations being made in Scotland. MacLeod (1938) approached this problem by testing the protection afforded by arsenic dips against experimental myiasis; during late autumn and winter the period of protection lasted for 3–4 weeks, with two soluble arsenic compounds. It should be noted that Moore analysed the outside of the fleece, whereas MacLeod's tests concern the skin or the extreme base of the fleece. The present results suggest that rain may penetrate the fleece of Welsh sheep to a certain depth below the surface. Thus, with yearlings dipped in winter and spring, when

the wool is fairly long, rain does not penetrate beyond the outer third of the fleece, judging by the analyses of the wool arsenic content. When lambs having short wool are dipped in summer, rain appears to enter the basal half of the fleece, though this does not necessarily mean that moisture reaches the skin itself. There were also indications that rain penetrates the fleece more readily if much suint is deposited in the wool with the dip. The penetration of the fleece by rain is obviously dependent on a number of factors, such as breed of sheep, various wool characteristics and climatic conditions. Little is known about the wettability of the fleece and the effect on this of wool constituents and of wetting agents added to the dip; this subject has an important bearing on the sheep maggot problem in relation both to susceptibility and control, as a low humidity at the base of the fleece is the surest protection against maggot infestation.

Summary

Investigations were made on the effect of wetting agents on arsenical sheep dips; it was found that the presence of a wetting agent leads to a decreased retention of arsenic in the wool and to poorer protection against artificial maggot infestation. Laboratory experiments are described on the effect of wetting agents on the amount of water retained by the wool after immersion; also, on the effect of auxiliary materials on the resistance of arsenic in dipped wool to leaching by water. The soap present in the suint, the water soluble fraction of the fleece, accumulates in the bath and markedly affects the physical properties of the dipping fluid; thus, the surface tension progressively falls as sheep pass through the dip. The effect of suint on the behaviour of sheep dips is discussed with special reference to blow-fly attack. An investigation was made of the distribution of arsenic along the staple and over the body following dipping. The leaching of soluble arsenic by rain appears to be confined to the outer third of the fleece in Welsh sheep in late winter and spring; in summer, with lambs having a short fleece, rain removes some soluble arsenic from the basal half of the fleece.

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SHEEP BLOW-FLY INVESTIGATIONS

X. ON THE POSSIBILITIES OF CALOMEL FOR THE CONTROL OF SHEEP MAGGOTS

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(With 1 text-figure)

Various workers in Australia and Great Britain have investigated the problem of controlling sheep maggot infestation by chemical means. Although new materials have been tested, attention has been mainly devoted to larvicides, repellents, and antiseptics; the use of an ovicide does not appear a likely method at first sight. Blow-fly eggs are laid in compact clusters, usually towards the base of the fleece where they hatch in a few hours owing to the high temperature; consequently, a toxic gas is the only substance likely to penetrate the clusters and kill all the eggs in the brief time available. In order to protect sheep for a reasonable period, it would be necessary to use a material which continues to evolve a toxic vapour for several weeks. It will be shown that calomel fulfils these requirements. The present work was started at the suggestion of Mr D.W. Wright who informed me in a private communication that he had found the vapour from calomel toxic to blow-fly eggs. Calomel has proved successful for the control of the cabbage root fly (*Delia* (*Hylemyia*) *brassicae* Meig.), the insect being killed in the egg stage (Wright, 1940).

Experimental

Materials and methods

The type of calomel used in most of these experiments was a proprietary brand supplied by the Leyton Manufacturing Co. Ltd. and known as 'Lemolac'; this is a very fine powder which forms a good suspension in water after preliminary pasting. Some experiments were also carried out with a sample of colloidal calomel supplied by the same firm. Although calomel is a very heavy chemical, it was not found necessary to add a suspending agent as the agitation of the dip by the sheep kept the powder in suspension. The calomel dust used in these experiments contained 4 % of calomel.

Ovicidal action was tested under laboratory conditions in two ways. Method A was used only for examining the toxicity of wool samples. The sample was placed in a 3 × 1 in. glass tube and moistened, eggs of *Lucilia sericata* being exposed over the wool in a small glass vessel; the tubes were then stoppered and incubated at 23° C. Control tests were carried out to confirm that the eggs were fertile. Method B was more critical; the eggs and the test material were placed in separate 3 × 1 in. tubes inside a stoppered glass bottle of about 400 ml. capacity. The experiments were carried out at 23° C. and a little water was added to each bottle to maintain the humidity. By a combination of these two tests, a method was worked out for assessing the toxicity of wool samples to eggs. Ovicidal action under field conditions was tested by placing eggs in the fleece and observing whether hatching occurred. As the eggs rarely hatched in the fleece of untreated sheep, a small piece of wet cotton wool was placed close to the eggs and the outside of the fleece was drenched with water. For all these tests, the eggs were collected within 1 hr. of oviposition.

With regard to the sheep used in these experiments, the expression 'lambs' refers to lambs aged 3–5 months; 'yearling' will be used to designate sheep born in the previous season and aged 9–15 months.

Preliminary dipping tests

Preliminary experiments showed that calomel renders the fleece toxic to blow-fly eggs for a considerable period. Sheep were dipped in a suspension of calomel and wool samples were collected at weekly intervals, ovicidal action being tested by method A. In the first experiment, yearlings were dipped in spring with 0·2 % calomel; it was found that the wool remained toxic to eggs for 6 weeks. In the second experiment, lambs were dipped with 0·1 % calomel in summer; in this case the wool remainded ovicidal for 4 weeks. In view of these promising results, a more detailed investigation was started.

Chemistry of the ovicidal action of calomel

The ovicidal effect of calomel is due to a toxic gas, contact being unnecessary, and it seems probable that the active principle is mercury vapour. In a review of the toxicity of this substance to insects, Gough (1938) concluded that it is the egg stage which is most seriously affected. In the present investigation, mercury vapour killed *L. sericata* eggs but had no effect on first instar larvae; similar results were obtained with the vapour from wool treated with calomel. Unsuccessful attempts were made to follow the chemical changes which occur when wool is treated with calomel; mercury compounds are difficult to analyse especially in the minute amounts involved in ovicidal action. However, a study of the process by biological means threw some light on the problem. Although wool treated

TABLE 1. *Effect of various substances mixed with calomel on* L. sericata *eggs*

Calomel alone	+	1 % sodium bicarbonate	−
4 % calomel dust	−	calcium carbonate	−
Calomel:		calcium arsenite	− (B)
+3 % suint	−	zinc arsenite	−
+crude wool	−	zinc arsenate	+
+washed wool	−	zinc oxide	+
+wool grease	±	arsenic trioxide	+
+1 % potassium chloride	+	arsenic dip	− (B)
+10 % potassium chloride	−	sodium thiosulphate	− (B)
+10 % dextrin	+	charcoal	−
+1 % sucrose	+	zinc dust	−
+1 % glucose	+	linseed oil	−
+sulphur	+	olive oil	+
+kaolin	+	oleic acid	+

\+ Eggs hatched.
− No eggs hatched (i.e. calomel activated).
± Small proportion of eggs hatched.
(B) Calomel blackened owing to separation of mercurous oxide and/or mercury.

with calomel is toxic to *L. sericata* eggs, it was found that a paste of calomel and water had no effect when tested in 400 ml. bottles (method B); this showed that wool 'activates' calomel. Further tests were, therefore, carried out with various wool components and other substances. Insoluble materials were mixed with calomel in equal proportions and made into a thick paste with water; for soluble substances, calomel was pasted with a solution. The ovicidal action was then tested in bottles and the results are shown in Table 1.

Crude wool and various fractions of wool render calomel toxic to *L. sericata* eggs. Chlorides and carbohydrates were tested as they are stated in the literature to accelerate the decomposition of calomel. Chlorides were effective at a high concentration but various carbohydrates proved inactive. Generally speaking, the substances found to activate calomel are either reducing compounds, such as zinc dust and sodium thiosulphate, or compounds which neutralize acidity; for example, calcium carbonate and sodium bicarbonate. Strongly alkaline materials, such as arsenic-sulphur dip, cause the calomel to darken owing to the separation of mercurous oxide and mercury. The failure of arsenious oxide to activate calomel may have been due to its feeble acidic properties. This method of investigation was used primarily for studying the action of calomel in relation to sheep dips, but the same technique might prove useful for studying the interaction between calomel and soil in connexion with horticultural problems. A sample of kaolin proved ineffective, whereas the filler in the 4 % calomel dust activated calomel; this material is stated to be a high silica clay.

Effect of temperature and humidity

A few experiments were carried out on the influence of these factors. As might be expected, calomel seems to be more effective at high temperatures; thus, calomel paste alone sometimes proved ovicidal at 37° C., whereas it was repeatedly found inactive at 23° C. On the other hand, samples of dipped wool, which had been found to kill eggs at 23° C., gave uncertain results when tested below 20° C. The fleece temperature would not fall below this figure in summer except on the outside; *L. sericata* usually lays its eggs in the middle or basal part of the fleece and is not as a rule active at temperatures below 20° C. Eggs of this species are unsuitable for testing the effect of humidity as they are unable to hatch under dry conditions. Preliminary experiments suggested that wool dipped with calomel was ineffective at 70 % relative humidity; this was later traced to the use of sulphuric acid for controlling humidity as the same material proved effective at this humidity when a potash solution was used in place of sulphuric acid. Apparently the acid absorbs the toxic vapour.

Results of small scale dipping experiments

For studying the persistence of ovicidal action in the fleece and the effect of the concentration of the dip, it was necessary to assess the ovicidal properties of wool samples. Table 2 shows the arbitrary method of evaluation which was adopted.

TABLE 2. *Method of assessing ovicidal properties of wool samples*

	Arbitrary grading
No effect on eggs in small tube	0
Killed eggs in small tube, but not in 400 ml. bottle	1
Killed 90–99 % eggs in bottle	2
Killed 100 % eggs in bottle	3
Grade 3 samples were in some cases further differentiated:	
Killed <50 % eggs after 4 hr. exposure	3*a*
Killed >50 % eggs after 4 hr. exposure	3*b*

TABLE 3. *Ovicidal properties of wool samples from sheep dipped in calomel*

	Days after dipping		
Dip	2	12	27
0·4 % calomel	2, 3, 3	3*a*, 3*b*, 3*b*	3*b*, 3*b*, 3*b*
0·15 % calomel	3, 3, 3	3*a*, 3*a*	3*a*, 3*a*, 3*b*
Ditto + 0·15 % dextrin	2, 3, 3	3, 3, 3	1, 2, 2

For significance of figures, see Table 2.

Yearlings in fairly full fleece were dipped in different mixtures in March, wool samples being collected at intervals after dipping. The results of the ovicidal tests are shown in Table 3. One lot of sheep was dipped with a mixture of calomel and dextrin; it was thought at the time that dextrin might increase the effect of the calomel, the laboratory experiments on activation not having then been carried out. The addition of the dextrin led to a decrease in ovicidal action at the end of a month, and this may be explained by the observation that dextrin decreases the retention of liquid by wool (Hobson, 1941). When calomel alone was used, there was very little difference between sheep dipped in 0·4 % and 0·15 %; also, the ovicidal properties of the fleece were if anything slightly greater after 27 days than after 12 days. It has been shown, however, from the arsenic content of wool, that sheep in full fleece retain a considerably higher proportion of dip in the wool than do lambs with a short fleece (Hobson, 1941). A further dipping experiment was therefore carried out in July with five groups of three lambs. Laboratory tests were made on wool samples as before; in addition, one group of lambs was tested in the field, eggs being placed in the fleece.

If the lambs dipped in 0·2 % calomel are considered, it will be seen from Table 4 that the ovicidal action of the wool persisted for 5 weeks, judging by the laboratory tests. Under field conditions (Table 5), the ovicidal properties broke down in the fourth week. This shows that the laboratory tests, carried out under closed conditions, are not sufficiently critical; nevertheless, this method is useful for comparing different preparations. If the amount of calomel in the dip is reduced, the ovicidal

properties of the wool are decreased and this is especially noticeable in the later series of samples (Table 4). Colloidal calomel proved no more effective than the standard calomel, nor was the ovicidal action improved by the addition of zinc arsenite, which had been shown to activate calomel (p. 274); the latter result is probably due to the fact that sheep's wool itself activates calomel. Since calomel is an expensive chemical, the question of the correct strength is an important one. These dipping experiments suggest that calomel is unlikely to prove effective under natural conditions at less than 0·2 %, and this concentration was therefore used in the field experiments which will be described later.

TABLE 4. *Ovicidal properties of wool samples from sheep dipped in calomel*

	Time after dipping		
Dip	2 weeks	3 weeks	5 weeks
0·2 % calomel	3, 3, 3		2, 3, 3
0·1 % calomel		2, 3, 3	
0·05 % calomel	2, 2, 3		0, 0, 1
Ditto + 0·2 % zinc arsenite	1, 2, 2		1, 1, 1
0·05 % colloidal calomel	1, 1, 3		0, 0, 2

For significance of figures, see Table 2.

TABLE 5. *Results of field tests on ovicidal properties of the fleece*

Days after dipping	Lambs dipped in 0·2 % calomel	Controls
3	+ −	+ +
4	− − −	− − −
8	− −	+ +
12	− − −	− − −
14	− −	+ −
18	− − −	− − −
21	+ − −	+ +
29	+ + +	+ + +

\+ Eggs hatched. − Eggs failed to hatch.

TABLE 6. *Results of myiasis experiments with lambs dipped in* 0·1 % *calomel*

Days after dipping	Dipped lambs	Controls
16	− − −	+ + −
19	− − −	+ + −
21	± −	+ −
23	+ ±	
25	+ −	+ − −
Proportion of positive results	2/12	6/11

\+ Maggots developed and produced wound.
± Maggots alive, but no wound.
− Maggots dead.

Larvicidal action of calomel

Although first instar larvae were not found to be affected by the vapour from calomel, this substance proved toxic when fed to larvae. Calomel is not poisonous to animals, as a rule, on account of its insolubility; its toxicity to blow-fly larvae is probably the result of chemical changes brought about by the alkaline reaction in the gut of these insects. This problem is being further investigated. The results of myiasis experiments on lambs dipped in 0·1 % calomel are given in Table 6.

As the lambs used in this investigation had a short fleece, the usual method of producing myiasis (Hobson, 1940) was not successful. Normal sized plugs of cotton wool fell out of the fleece and small plugs tended to dry up; the method adopted was to place the eggs on the skin under small moistened plugs and spray the outside of the fleece with water. Although the proportion of successful experiments among the controls was low, these results suggest that calomel afforded some protection against

the development of maggots, but this was not due to ovicidal action as eggs on the point of hatching were used. The calomel may have been acting as a stomach poison or exerting some effect on the sheep's skin; thus, it was noticed that the skin was not so highly inflamed as usual when live maggots were found.

Ovicidal properties of other sheep dips

The immediate ovicidal properties of calomel and proprietary dips were tested by placing egg clusters in sheep's wool soaked in the dip. Carbolic dip at normal strength and 0·1 % calomel proved toxic to *L. sericata* eggs, but an arsenic-sulphur powder did not kill eggs, even at five times normal dipping strength. This shows that dipping with arsenic will not prevent the hatching of eggs already in the fleece; it is also possible that the young maggots may survive if the dip has not dried, since experiments have shown that arsenic solutions have no effect as contact insecticides on maggots. This is a point of some importance as maggot infestation does sometimes occur 3–4 days after dipping in arsenic and this could probably be avoided if an ovicide were added to the dip. Calomel is unsuitable for this purpose as it is decomposed by alkaline solutions and it is also incompatible with carbolic dips since it breaks the emulsion.

Field experiments with calomel as a fly dip

In order to test the performance of calomel under field conditions, extensive dipping experiments were carried out during 1940. For each experiment, the sheep were divided into two equal groups and kept in the same field; if the sheep were of different types, the two groups were similarly constituted.

TABLE 7. *Comparison of calomel and arsenic-sulphur dips*

		0·2 % calomel		Arsenic (full strength)	
Farm		No. dipped	No. infested	No. dipped	No. infested
Madryn	18. vi. 40	91 lambs	1	91 lambs	4
Llysfasi	19. vi. 40	48 lambs	3	49 lambs	6
Madryn	7. viii. 40	79 lambs	13	79 lambs	3
Mostyn estate	22. viii. 40	66 lambs 211 ewes	1	70 lambs 190 ewes	0
Llysfasi	23. viii. 40	71 lambs 96 ewes	9	68 lambs 84 ewes	17
Tynllan	30. viii. 40	36 lambs 82 ewes	1	36 lambs 82 ewes	2
	Total	780	28 (3·6 %)	749	32 (4·3 %)

When the sheep available for experiment on one farm were grazing in different fields, each lot was divided into two. One group was dipped in 0·2 % calomel, an arsenic-sulphur dip at full strength was used for the control group, the farmer using his usual brand of dip. The shepherd kept a daily record of maggot infestation for 5 weeks following dipping. As the conditions in each trial were as nearly as possible identical, the results could be added together without taking percentages, which are misleading with small groups of sheep. Table 7 gives the details of these experiments; Fig. 1 shows the aggregate number of strikes which had occurred at different times after dipping.

As the rainfall was low at the time of these experiments, the sheep were not infested with maggots to the same extent as in normal seasons. Most of the strikes occurred in the tail region as a result of soiling of the wool with dung or urine. The total figures indicate that, for preventing maggots, there was little difference between the calomel and arsenic dips. This result is encouraging as it suggests that the protective effect of calomel equals that of a mixture of arsenic and sulphur, which is the popular dip in Great Britain for preventing maggot infestation. The most striking feature revealed by the graph is the short period of complete protection afforded by both dips against tail strike, about 10 days.

Examination of lambs dipped with calomel showed that the skin retained its normal healthy colour and did not become inflamed. The wool assumed a greyish tint and shepherds on lowland farms commented favourably on the appearance of the fleece. The grey colour may be a disadvantage on hill farms as the sheep cannot be spotted so readily, but this could be easily overcome by the addition of bloom to the dip.

Experiments with 4 % calomel dust

Calomel appears to be especially suitable as an ingredient of dusts, since it acts over a distance and contact is not necessary. In order to find the best means of applying calomel dust, three groups of two lambs were treated with this material in different ways, 1½ oz. of dust being spread over the back of each lamb and worked into the fleece by pressing the hand along the back. Wool samples were collected 9 days later and their ovicidal action assessed as in the case of the dipping experiments. The home-made 'pepper-pot' consisted of a tin with holes punched in the lid. Table 8 shows that the 'pepper-pot' method gave the best results, though the differences observed were not great. For dusting the tail region, the hand bellows are more convenient as puffs of dust can be directed upwards.

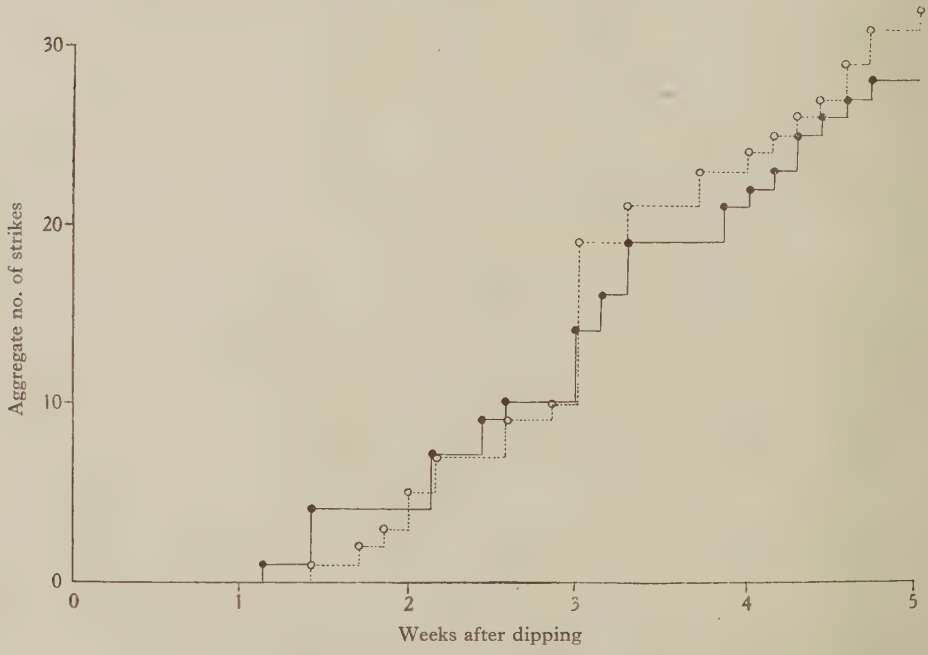

Fig. 1. Different times after dipping at which strikes occurred.
Full line calomel group; broken line arsenic group.

TABLE 8. *Ovicidal properties of wool samples from lambs treated with 4 % calomel dust*

Method of dusting	
Mechanical duster	2, 2
Hand bellows	1, 3
'Pepper-pot'	2, 3

For significance of figures, see Table 2.

Field experiments were carried out in 1939 on the effect of calomel dust on maggot infestation. The technique was the same as that used in the dipping experiments, a direct comparison being made between two groups of sheep in equal numbers under identical conditions. The control group was left untreated; the other group was dusted by means of hand bellows. 2 oz. of calomel dust were applied over the back and around the tail of each lamb, especial attention being paid to the tail region and to the shoulders. The details of these experiments are given in Table 9.

In the 1939 trials, calomel dust gave good results at farms A, C and D, where the infestation was relatively low among the controls, but failed at farm E where infestation was high. Another dusting experiment was carried out at this farm in 1940 and again the dust gave poor results. The proportion of lambs struck was very high and enquiry revealed that the shepherd had included restrikes (i.e. reinfestations of maggot wounds) in these figures. Many of the lambs were badly marked and the opinion was formed that the severity of attack was partly due to poor shepherding. The experiments at this farm, therefore, were not altogether satisfactory. Further evidence is needed before the value of calomel dust for this purpose can be judged; the results so far obtained suggest that dusting gives satisfactory control if the conditions are not too favourable for maggot infestation.

TABLE 9. *Effect of calomel dust on maggot infestation*

		Dusted group		Untreated group	
Farm	Year	No. lambs	No. infested	No. lambs	No. infested
A	1939	15	0	15	5
B	,,	49	0	49	0
C	,,	11	0	11	2
D	,,	23	0	24	5
E	,,	25	7	25	10
E	1940	30	17	27	18
	Total	153	24	151	40

Period of observation 5–6 weeks after dusting.

DISCUSSION

The present investigations have shown that the application of calomel to the fleece renders it toxic to blow-fly eggs for an appreciable period. They also suggest that calomel will prove a valuable addition to the chemicals available for the control of sheep blow-flies. Calomel is a relatively expensive chemical and this may limit its use in dips; its chief value may be as an ingredient of protective dusts and maggot dressings.

With regard to the use of calomel as a dip, the field experiments indicate that a 0·2 % suspension of calomel protects sheep against maggots to about the same extent as do arsenic-sulphur dips. The *advantages* of calomel are that it is less poisonous to animals than arsenic, acts over a distance and does not produce the scalding of the sheep's skin, which frequently follows dipping with arsenic and leads to a scabby condition at the base of the fleece; this is an important point as the scabby condition may render the sheep susceptible to maggots when the chemical immunity due to arsenic has disappeared. Arsenic acts mainly as a larvicide and is only effective at the extreme base of the fleece, where the amount of poison decreases owing to the growth of new wool (Hobson, 1941); this factor will not affect calomel to the same extent since its action is due to an ovicidal vapour. The *disadvantages* of calomel are its high price and its inability to kill pests such as keds, ticks and lice. No observations have been made on the effect of calomel on external parasites, but it seems probable on *a priori* grounds that calomel will not kill the adults though it may control these pests (with the exception of ticks) by killing the eggs; this may apply even in the case of keds, in which the development of the eggs and larvae occurs within the body of the female, since the toxic vapour may reach the eggs via the tracheal system of the female. However, the shepherd naturally expects to see a summer dip *kill* any parasites in the fleece, especially keds; this drawback of calomel might be obviated by the inclusion of derris, but this would

increase the cost, which is already a limiting factor. The writer discussed the matter with dip manufacturers, who pointed out that it would be an expensive matter to change over from the preparation of arsenic to that of a substitute and that this was not likely to be undertaken unless the substitute had been shown to be definitely superior to arsenic. Since in the field tests the two dips gave similar results, it seems unlikely that calomel will oust arsenic as a summer dip.

Although dipping is by far the most convenient method of impregnating the fleece with a protective chemical, dusting is preferred on some farms. The chief difficulty with a maggot dust is to get the material through the wool down to the skin. This is not so important in the case of calomel since the toxic vapour can spread through the fleece; also the eggs, although pushed down into the wool, are not usually laid at the extreme base of the fleece. Calomel, therefore, seems to be the ideal insecticide for a maggot dust. Calomel dust might be used in several ways. For the general protection of sheep against maggots, the dust can be applied around the tail, over the back, and to the ribs if deemed necessary. On lowland farms where the sheep are examined daily, dirty sheep might be dusted after cleaning them. Even when the soiled wool has been shorn away, the skin often remains sticky and attractive to flies; calomel dust would dry the skin and kill any eggs that are laid. This dust might also be used in the treatment of infested sheep.

After maggot wounds have been dressed they are liable to become reinfested later, especially in wet weather; since arsenic cannot be applied to open wounds, it is a difficult matter to devise a dressing which will protect the wound for more than a few days. Calomel should prove useful for this purpose; it can safely be applied to wounds, is mildly antiseptic and renders the wool ovicidal for a considerable time. One method is to treat the wound first with a suitable maggot dressing and finish off by applying calomel dust to the raw surface and to the wool around the wound. However, as the majority of struck sheep are treated in the field, a better plan would be to incorporate calomel in the dressing. There are certain technical difficulties about this which are being investigated; the majority of dressings are emulsions and the addition of calomel breaks the emulsion.

Summary

When sheep are treated with calomel, either by dusting or by dipping, the fleece is rendered toxic to eggs of the sheep blow-fly; the active principle is volatile and appears to be mercury vapour. Under the conditions used in the laboratory tests, calomel alone does not kill blow-fly eggs; wool and its components, also various other substances, 'activate' calomel. The possibilities of calomel as a maggot dust and as a dip have been examined, both by small scale tests and by field experiments. It is concluded that calomel is likely to prove a useful insecticide for the control of sheep blow-flies, particularly as an ingredient of protective dusts and maggot dressings; the high price of calomel may limit its use as a dipping material.

I am indebted to the Agricultural Research Council for a grant which has financed this work. My appreciations are also due to the Leyton Manufacturing Co., Ltd., for supplies of calomel and for helpful advice. I take this opportunity of expressing my gratitude to the

owners of the sheep and to the shepherds for their co-operation in the field experiments. Finally, I wish especially to thank Mr D. W. Wright, of the Horticultural Research Station, Cambridge, for suggesting this line of work to me, and for informing me of his results prior to publication.

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WIREWORM POPULATIONS AND THEIR EFFECT ON CROPS

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(With 1 Text-figure)

1. Introduction

The objects of the scheme of investigations on wireworms, begun in 1939 by the Advisory Entomologists of the thirteen Agricultural Provinces of England and Wales, in co-operation with the Ministry of Agriculture's Plant Pathological Laboratory and the Statistical Department of Rothamsted Experimental Station, have been stated in the Interim Report (Anon. 1940) as:

'(*a*) To find a method of estimating the wireworm population of a grass field with sufficient accuracy and sufficient speed to be of service in advisory work.

'(*b*) To discover any obvious correlations between the size of the wireworm population in any field and other factors such as soil type or cultural treatment.

'(*c*) To obtain some guide as to the damage to be expected from wireworm populations of different sizes.'

The 100 fields of grassland about to be ploughed, which were examined during the first season, provided information on (*a*), on the basis of which improvements in the sampling technique were recommended, but the records were neither in numbers nor in content sufficient to do more. In consequence, an extended scheme of observations was designed for the Wireworm Survey of the 1939–40 harvest year. In addition to the estimation of infestation, and to estimation also of plant density and yield of the first crop after grass, this scheme embraced the recording of many other details relating to the history and character of the field and the crop. With these last it is not proposed to treat here, though they are clearly the data relevant to (*b*) above.

The first part of this paper, §§ 2–5, contains a discussion of (*a*) and of issues arising therefrom, making use of the records of nearly 500 fields. The information on plant population and on yield enables an investigation of points raised by (*c*) also to be made, and this comprises §§ 6–8. Unfortunately, the evidence available for this study is only sufficiently full for oats, and the discussion of other crops must await a further extension in the number of records. The term 'wireworm' as used in this report generally refers to the three common species of *Agriotes*, with *A. obscurus* predominating; occasional specimens of *Athous haemarrhoidalis* and of other species have been found and included in the counts without comment, their number being too few to have any bearing on the results.

2. The sampling technique

For the wireworm sampling undertaken during 1939 the standard size of sample was a 6 in. square of soil; samples were to be taken to the greatest depth at which wireworms could be found, for which purpose most observers seem to have been satisfied with a depth

of 6–9 in. As a result of these preliminary investigations, it appeared safe to recommend that in future a cylindrical core of 4 in. diameter should be used as the standard sample. If the same number of samples is taken there is only one-third of the volume of soil to be examined, a gain which outweighs the loss of information due to the lesser accuracy of the smaller samples. Equal precision in the estimation of populations will require approximately twice as many of the small samples as of the large. The area of a 4 in. core is almost exactly one five-hundred-thousandth of an acre, and thus the conversion of sample counts to estimated populations per acre is very simple.

It was advised that twenty samples per field should be taken, two being located randomly in each tenth of the field. This plan was chosen so as to allow a comparison to be made of the variation between and within the ten sections of the field. This number of samples was actually taken from most of the fields sampled, but in some cases only ten were taken, and instances occurred of other numbers up to a total of twenty-six samples. The arrangement of the samples in pairs was not always followed, a random selection of sampling points over the whole fields being sometimes used. Some observers in 1940 still preferred to use the 6 in. square as the sampling unit, and cases occurred of individual fields being sampled with 8 in. squares or with 3 in. cores, but the 4 in. core was the unit generally adopted.

It is recognized that the figures for the numbers of wireworms per acre used in subsequent sections do not represent the total populations present. The technique adopted for counting had necessarily to be sufficiently rapid to be of use in advisory work and it is known that the smaller larvae (below 8 mm. in length) were not exhaustively counted. Though these probably form a large part of the total population, it is evident from the results discussed in §§ 6 and 7 that the numbers of the larger wireworms, which are considered to have been almost fully ascertained by the technique used, were a good indication of the crop damage to be expected in the harvest year after sampling.

Records of samplings from 473 fields were received during the 1940 season. Most of the sampling took place before ploughing, either in the autumn of 1939 or in the late spring of 1940, and the few records from fields sampled after ploughing, either before or after sowing, can scarcely bias the general conclusions.

3. Geographical distribution of wireworm populations

From an examination of the mean populations of the 473 fields sampled, there can be little doubt that wireworm infestation is considerably higher in the south of the country than in the north. The means for the thirteen Provinces are set out in Table 1, together with the percentage of fields having populations of more than 300,000/acre, a figure chosen as being one below which little damage to oats and wheat was found.

Differences between provinces might in part be the result of the conditions of counting the samples rather than of true differences in infestation. Examination by hand must inevitably introduce a personal element and the difficulties of discovering the smaller wireworms in a sample will be much greater in heavy than in light soil. Also it is known that some observers examine their samples in the field and others prefer to make their examination in the laboratory. Moreover, the fields sampled in any province are not necessarily fully representative of that province; in some instances almost all the fields were from a single county. Nevertheless the geographical trends in mean population appear too regular

to have been the chance result of any of these causes. The provinces have been grouped into three regions, south, west, and north, there being a distinct break in the sequence of mean populations between the lowest province of one region and the highest of the next. It is arguable that, in view of their exceptionally high infestations, the south-eastern and southern provinces should constitute a separate region, but, for the study of the relationship of infestation and crop there were too few fields available for this separation to be profitable. More extensive information on these differences should be obtained from the sampling of the 1940–1 season, as a result of which it should be possible to examine in greater detail the grouping of provinces, or even of counties.

TABLE 1. *Wireworm infestation in the thirteen provinces*

Province	No. of fields	Mean population in 1000/acre	% fields with more than 300,000/acre
South-eastern	82	1025	93
Southern	33	824	88
Eastern	50	627	72
Midland	42	540	62
Western	16	520	62
West midland	38	505	53
South Wales	29	394	59
South-western	35	327	43
Mid-Wales	17	308	24
Yorkshire	30	234	30
North Wales	30	225	27
North-western	40	208	8
Northern	31	133	10
South (total)	261	738	75
West („)	81	347	44
North („)	131	200	18
All	473	522	54

4. PATCHINESS WITHIN THE FIELD

It is sometimes argued that patchy distribution of wireworms within a field invalidates the use of an average figure for the population of the field. Cases are instanced of fields which are heavily infested at one end and almost free from wireworms at the other, for which any cropping recommendation based on the average population may have unfortunate results. Without prejudice to the use of more detailed recommendations in such cases, the general applicability of this criticism to all wireworm sampling may be tested by the comparison of the variability of populations between and within sections of the same field.

After rejection of fields with less than sixteen samples in order to ensure satisfactory estimation of the variabilities there remained 396 fields for which this comparison could be made. An analysis of variance for 'between' and 'within' sections was carried out on the counts from each field. The fields were then classified into three groups according to the ratio of the mean squares, the ratios separating the groups being taken as 1·5 and 3·0 (see Table 2). This ratio may be taken as a measure of the patchiness of a field, since it shows the extent to which variation between sections exceed intrasection variability. If the distribution of wireworms in a field were purely random, it would be expected that 27 % of all fields would be sufficiently patchy to show a variance ratio of at least 1·5, and 5 % of all fields would have a ratio greater than 3·0.

There is clearly no sign of any excess of fields above the number expected in the middle category, but, for the south and west regions only, about 12 % of all fields are found in the group of greatest patchiness instead of the 5 % expected. There is no evidence of any departure from randomness in the north. It seems then, that in the more heavily infested districts, there is some tendency for the larvae to be congregated in patches instead of randomly distributed over the field. This effect is however quite small, and can scarcely be of much practical use or importance; certainly it does not invalidate the general use of the mean population as the measure of infestation of a field.

Indeed for any use to be made in practice of intra-field variation in infestation, it would presumably be necessary for there to be areas of a field sufficiently different in population and suitable in shape for them to be cropped separately. Examination of the location on the field of the samples from patchy fields suggests that less than one-fifth of such fields fulfil these two conditions. It thus seems probable that only about 5 % of all fields have sufficiently regular and pronounced trends in their degree of infestation to make the dividing of the field for cropping worth consideration.

TABLE 2. *Patchiness of infestation*

Region	No. of fields with variance ratio			Total
	−1·5	1·5–3·0	3·0–	
South	141	40	24	205
West	51	15	11	77
North	90	16	8	114
All	282	71	43	396

Though this analysis has vindicated the use in general of a mean population, it should not be taken to mean that in no circumstances need the distribution of infestation within the field be considered. Undoubtedly cases do occur of very great differences in population between the two sides of a field, and it is always open to the adviser to make his recommendations accordingly, if necessary taking additional wireworm samples from each division of the field in order to form satisfactory estimates of the separate populations. A cause of patchy failure of a crop which is possibly more important than the occurrence of areas of high wireworm concentration is the greater effectiveness of wireworm damage in destroying the crop in areas where the young plant has also to combat poor fertility conditions. Such a situation is very likely to arise on land newly brought under the plough, and for it the wireworm can scarcely be held directly responsible.

5. Estimation of sampling errors

Using the ordinary statistical technique, a standard error per sample was computed for each field. This was taken from the total variation of all samples and not from the 'within sections' mean square, as, in view of the evidence described in the preceding section, the former seemed in general to be the best available estimate. Considering for the present only those fields sampled by 4 in. cores, and rejecting a few on account of insufficient sampling, there remain 309 fields for the study of the sampling errors and their relation to the mean infestation.

When the average number of larvae per sample was small (less than 0·5, corresponding

to 250,000/acre) the distribution of the number found per sample was satisfactorily fitted by the Poisson Law; that is to say the sampling variance was equal to the mean per sample. For denser populations, however, the variance was higher than would result from the Poisson distribution. For each of the 309 fields the percentage standard deviation (coefficient of variation) was calculated and plotted against the mean count per sample. Though the points thus obtained were very variable, they indicated a decrease with increasing population which was less rapid than the Poisson Law would predict; when the mean count becomes high—of the order of 4·0—the percentage standard deviation is decreasing very slowly and is almost constant at about 80 %. The mean values of the ratio in successive small ranges of the mean count were computed and a smooth curve was drawn by eye through the points obtained. This curve, hereafter taken as representing the relationship between mean count and proportionate sampling error, is shown in Fig. 1. In this figure the curve for a Poisson distribution is also drawn, but the individual observations have not been shown, as the points are in many places very congested.

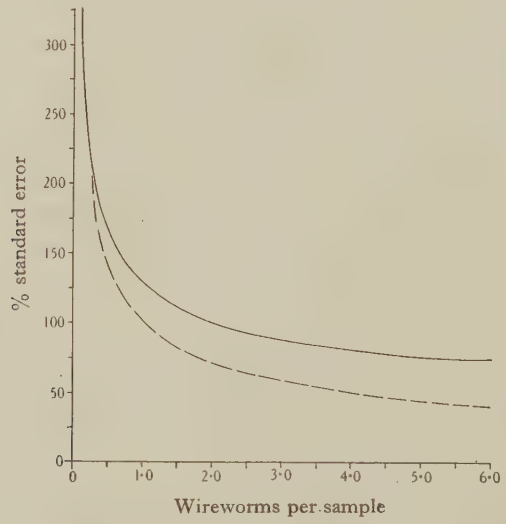

Fig. 1. Relationship between numbers of wireworms per sample and the sampling errors for a 4 in. core of soil. ———— Estimated from 309 fields. — — — Poisson distribution.

From the curve the sampling error corresponding to any population may be read. For example a population of 500,000/acre, averaging 1·0 larva per sample, has a sampling error of 126 % or 630,000/acre. The standard error of a mean population calculated from twenty samples is therefore 141,000/acre. Using a table of normal deviates, limits may be determined beyond which an observed mean of twenty samples will lie with a given probability. Thus the normal deviate for a 12·5 % probability is 1·150 and deviations of 162,000/acre or more will occur with a probability of 12·5 % at each extreme. That is to say, of fields having true populations of 500,000/acre, one-eighth will, on account of sampling variation, appear to have populations less than 338,000 and one-eighth more than 662,000 as a result of a sampling by twenty 4 in. cores. A series of results of this type is given in Table 3.

An alternative, and in practice more useful, aspect of the same results is shown in Table 4. The precise meaning of this table of fiducial limits needs careful thought, but may be briefly described as follows. From Table 3, a field population of 300,000/acre will be estimated by sampling to be greater than 418,000 once in eight times; similarly a population of 400,000/acre will once in eight be estimated greater than 541,000. By a linear interpolation, it is found that a true population of 367,000 will be estimated at a figure greater than 500,000 by one-eighth of the determinations. A similar interpolation shows that a population of 706,000/acre

TABLE 3. *Distribution of sampling means for populations of given density sampled by twenty 4 in. cores (thousands/acre)*

Density of population	$\frac{1}{8}$ of determinations less than	$\frac{1}{4}$ of determinations less than	$\frac{1}{4}$ of determinations greater than	$\frac{1}{8}$ of determinations greater than
100	40	65	135	160
200	105	144	256	295
300	182	231	369	418
400	259	317	483	541
500	338	405	595	662
600	416	492	708	784
700	495	580	820	905
800	578	670	930	1022
900	659	759	1041	1141
1000	739	847	1153	1261

A density of 100,000/acre corresponds to a total of 4 per 20 samples.

TABLE 4. *Probable limits of error of population values obtained from sampling by twenty 4 in. cores (thousands/acre)*

	Population value which would give a density			
	as great or greater than that observed		as small or smaller than that observed	
Observed density	in $\frac{1}{8}$ of the determinations	in $\frac{1}{4}$ of the determinations	in $\frac{1}{4}$ of the determinations	in $\frac{1}{8}$ of the determinations
100	—	—	144	192
200	130	154	264	323
300	204	239	380	452
400	285	327	494	579
500	367	415	609	706
600	449	504	722	827
700	531	593	834	951
800	613	682	947	1073
900	696	773	1059	1195
1000	781	863	1171	1313

One wireworm per twenty samples corresponds to 25,000/acre.

will give a density as small or smaller than 500,000 in one-eighth of the determinations. These two populations, 367,000 and 706,000/acre, are the lower and upper 12·5 % fiducial limits to an observed density of 500,000/acre. Fiducial limits for a series of observed densities up to 1,000,000/acre are shown in Table 4.

Similar tables may easily be constructed for any other number of samples per field. Tables for other sizes of sampling unit are not directly calculable from Fig. 1, but the results of the sampling by 6 in. squares in 1939 were used to obtain corresponding tables for that size of unit. For low population densities, where the Poisson Law operates, 6 in. squares are of

the same efficiency as 4 in. cores from the point of view of the precision of the estimates obtained, but when the density exceeds 500,000/acre, one 6 in. square is approximately equal in value to two 4 in. cores. Thus to obtain precision by 4 in. cores equivalent to that from 6 in. squares only one-third to two-thirds of the volume of soil need be examined, and the smaller sampling unit is in general to be preferred.

About twenty samples per field appears to be a satisfactory number to take in order to differentiate with reasonable assurance between heavy, medium, and light infestations. It has, however, been suggested that in areas of the country where the general level of infestation is low, small variations in population may be important in their effects on the crop. As will be seen from the ensuing sections of this paper, the present survey provides little evidence in support of this view, but if it should ever be necessary to practice a finer differentiation between these populations having proportionately high sampling variation, many more samples per field will be required.

Also, the sampling technique has so far only been tested for grassland, either before or immediately after ploughing. Until the sampling variations of fields sampled after cropping in 1940 have been examined, it is impossible to judge whether the same accuracy of estimation of populations can be obtained from 20 samples. It might be expected, for example, that there would be greater irregularity of distribution in a field of stubble than in grass, and that consequently more samples per field would be needed for adequate estimation, but there is not yet sufficient evidence on this point.

6. Plant density and wireworm population

Observers were asked to obtain, for as many as possible of the cereal crops, at least one sample determination per field of the plant density at an early stage of growth. The sampling technique employed was a slight modification of one that had been used for some years in sampling for yield, details of which have been described by Cochran (1938). The sampling unit was 2 ft. length of four adjacent rows (or, for a broadcast crop, an area 2 ft. square) and samples were taken at 30 or 60 yd. intervals in two lines along the field. Counts were recorded of all plants in each of the four rows of every sample.

The only crop for which any large volume of information has been collected in 1940 is oats; unfortunately the decision to take plant counts was made rather too late for many counts to be made on fields of wheat. At least one count was made on each of 183 fields of oats, and on 117 of these a second count was made one to four weeks later. Such second counts were to be made as late as possible, having regard to the increasing difficulty of distinguishing individual plants. The sources of sampling variation have not been examined in very great detail, but the figures for 41 counts from a single province indicate the chief variation to be between the two lines of sampling and that no very great gain would result from counting more rows per sample or taking more samples per line. The absolute values of sampling errors appear to be higher in the north, where plant densities also are higher, but, for a field of average size sampled according to the instructions, and thus having about 16–20 samples taken, the sampling error of the density computed from the mean count of the two lines may in general be taken as 15 % of the estimated density.

In considering the influence of wireworm infestation on plant density, it was decided to make use of the first plant count, rather than the mean, for fields on which two were taken. It was felt that this convention would bring the figures nearer to a true comparability having

regard to the development of the crop than would the use of an average count. The regression of plant count on wireworm population was then computed separately for the fields of each province. In order to obtain regression coefficients with a reasonable degree of precision, provinces were grouped once more into the three regions of § 3. The results are shown in Table 5.

A first point to be noticed about this table is the very much lower mean plant density in the south as compared with the other two regions. One cause contributing to this was probably the use of lower seeding rates in this part of the country. For the fields sampled in this group of provinces, 4–4½ bushels/acre was a normal seeding rate, whereas in Wales and the North of England seeding rates of 5–6 bushels/acre, and sometimes even higher, were employed. Only in the north was there sufficient variation in the rate of seeding for its effect to be judged, but 64 fields from this region show the results given in Table 6. The plant counts were, for the most part, taken soon after the appearance of the plants above ground, and, as would be expected at this stage, they showed considerable dependence on the amount of seed sown, the relationship being about linear.

TABLE 5. *Regression of plant density on wireworm population*

Region	No. of fields	Wireworm (1000/acre)	Plants (1000/acre)	Loss in plants per additional wireworm
South	63	710	920	0·34 ± 0·10
West	48	366	1230	0·49 ± 0·19
North	72	218	1310	1·22 ± 0·26
All	183	426	1160	0·46 ± 0·09

TABLE 6. *Influence of seeding rate on plant populations (north only)*

Seeding rate (bushels/acre)	< 5	5–5½	> 5½
No. of fields	9	38	17
Plants (1000/acre)	1050	1270	1560

Undoubtedly the chief interest of Table 5 lies in its clear indication of a progressive increase from south to north in the plant loss per additional wireworm. The most obvious explanation of this phenomenon is that when the wireworm population is low most of the plants killed are attacked by one wireworm only, but as the infestation is increased the chance of a wireworm finding an untouched plant is lessened and many plants will actually be attacked by two or more wireworms, thus reducing the average rate of plant damage per wireworm. The true relationship between infestation and plant density should therefore not be linear; this view is supported by plotting the two figures for each of the 183 fields. Inspection of the diagram gives no indication of consistent differences between regions, but only of a steady falling off in the linear regression with increasing infestation. A further test of this explanation was provided by a more detailed examination of the 63 fields in the south, which were subdivided into those with wireworm populations above and below 500,000/acre.

Though the precision of the regression coefficients in the two groups is less than those previously considered, the numbers of fields involved being small, it is noteworthy that the results for fields with less than 500,000 wireworms/acre agree very closely with those for the north region. In contrast, the damage per wireworm at the high level of infestation is very small. All the evidence thus supports the explanation suggested above.

For those fields on which two plant counts were taken, the interval between the two varied from a week to a month; in spite of the disturbance caused by this variation, it might be

expected that some relationship would exist between the loss in plants during the interval and the degree of wireworm infestation. Almost all fields showed a reduction in plant density, the average losses being 180,000/acre on thirty-two fields in the south, 240,000 on forty-two fields in the west, and 260,000 on forty-three in the north, there being approximately a 20 % loss in all cases. There was a slight indication that the loss was greater in the presence of higher infestations, the regression coefficient being $10{\cdot}3 \pm 5{\cdot}4$ additional plants lost for each additional 1000 wireworms. The smallness of this figure suggests that at the time when the first counts were taken, wireworms had already done their maximum damage in so far as the complete destruction of plants was concerned. Damage caused after this time—and in many cases in 1940 damage continued almost until harvest—might affect the development of the remaining plants but have little influence on their number. This explanation is not fully satisfactory, as it is difficult to understand why such a uniformly high loss of plants should have occurred as a natural competition effect in so short a period, particularly in view of the fact that, as will be shown in § 8, the stand in the south and west was already sufficiently below the optimal as to affect the yield adversely.

TABLE 7. *Plant density and wireworm infestation, south region only*

	No. of fields	Wireworm (1000/acre)	Plants (1000/acre)	Loss in plants per additional wireworm
High infestation	35	1060	760	$0{\cdot}15 \pm 0{\cdot}13$
Low infestation	28	272	1120	$1{\cdot}36 \pm 0{\cdot}64$

Records of wheat crops were almost entirely confined to the south region, and the only information of value on the wireworm—plant density relationship—is contained in fifteen fields from the South-eastern province. These had an average of 643,000 wireworms/acre and a mean plant density of 530,000/acre. The reduction in stand per additional wireworm was $0{\cdot}32 \pm 0{\cdot}16$ plants; though based on very few fields, the agreement with the average for the south region for oats suggests that the crops may have been about equally susceptible to wireworm attack. The wheat crops recorded were, however, almost entirely winter wheat, whereas the oats were nearly always spring sown.

7. YIELD AND WIREWORM POPULATION

Sampling estimates of yield were also obtained from a number of fields, the technique employed for cereals being the same as that for plant counts, the sample area being cut by hand and afterwards threshed. It was not the intention in this investigation to make any detailed study of sampling variation within the sampling lines, and therefore the samples were generally bulked for each line separately. The sampling error between lines was examined for ninety-eight fields of oats and indicated for the standard error of the mean yield of the two lines a value of about 15 % (or 3 cwt./acre for an average crop), a figure rather higher than had been anticipated.

As with plant density, oats is the only crop for which there are sufficient records for satisfactory conclusions to be drawn. The data were supplemented by yield estimates made visually by the observer or by the farmer, or alternatively from the threshing figures, so that yields for 147 fields in all were obtained. The regression of these yields on wireworm population was found in the same way as for plant density.

The results shown in Table 8 do not lend themselves to so straightforward an explanation as do those of Table 6, probably on account of the less direct influence of wireworm attack

on the ultimate yield. Nevertheless, they indicate that in the region of high infestations average yields were low by comparison with the rest of the country and the effect on yield of changes in the level of infestation was very considerable. The significance of this estimated loss of 1·2 cwt./acre for an additional 100,000 wireworms is undoubted. The apparent *increase* in yield with increasing infestation in the west is within the bounds of the errors of estimation; it seems justifiable to conclude that in neither the west nor the north was there much loss of yield due to wireworm at the low infestation levels normally found there, but there is no reason to suppose that the intensity of wireworm attack was any less than in the south on the few fields which were heavily infested.

Crops which were recorded as complete or partial failures, and were consequently redrilled (with the same or a different crop) or simply abandoned, have been excluded from the above analysis. In the north there were only two such fields of oats, and in the west only one, but failures were comparatively common in the south. The exclusion of all failures in this region will lead to the underestimation of the regression of yield on infestation since the higher

TABLE 8. *Regression of yield on wireworm population*

Region	No. of fields	Wireworm (1000/acre)	Yield (cwt./acre)	Loss in yield per 100,000 wireworm
South	50	574	18·8	1·17 ± 0·36
West	38	397	24·0	−0·40 ± 0·52
North	59	212	22·6	0·45 ± 0·42
All	147	383	21·7	0·38 ± 0·25

TABLE 9. *Oats yield and wireworm infestation; south region only*

No. of fields	Wireworm (1000/acre) Range	Wireworm (1000/acre) Mean	Yield (cwt./acre)
15	– 300	164	23·5
18	301– 600	449	19·0
16	601–1000	806	13·6
15	1001–	1328	8·7
64	All	641	16·7

infestations, at which most of these failures occur, will show too small a proportion of low yields. On the other hand, it is likely that all, or almost all, cases of failure were ascertained, whereas only on approximately half the fields of oats initially recorded were yield figures obtained. Hence to include all failures as having zero yields would be to give them undue weight. Assuming this 50 % ascertainment of yields, unbiased results may be obtained by giving half-weight to all crops recorded as failures. This has been done for Table 9, in which mean yields are given for the south region in four ranges of wireworm population, complete failures being recorded as zero yield and partial failures conventionally as 5 cwt./acre.

Table 9 shows very clearly the steady drop in yield with increasing level of infestation. From the extreme entries, it is seen that the average fall is 1·27 cwt./acre for each additional 1000 wireworms/acre. The removal of the bias from the results of Table 8 has thus slightly increased the estimate of the rate of yield loss.

These considerations of bias do not apply to the analysis of plant density made in § 6 above, since failures in general took place after the date at which the first plant counts were taken and there was therefore no similar bias in the selection of fields for which plant densities were recorded.

By making use of the observer's description of crops for which no numerical estimate of yield was obtained, it was possible to augment the crops available for study by an additional 64. Taking an average crop as 15·8 cwt./acre (Ministry of Agriculture's average for England and Wales, 1929–38), crops heavier than two-thirds average were classified as successful, those below one-third as 'failed', and those intermediate as 'poor'. The assignment to these classes may have been influenced by subjective judgement for borderline cases, but the number of crops for which there was any doubt is too few to have biased Table 10 to any appreciable extent.

Table 10, for which the author is indebted to Mr J.C.F. Fryer, shows in striking fashion the increasing chances of failure as the infestation increases. The entries in Table 10 have been further subdivided by regions, but little additional information is obtained, the west and north being very poorly represented among the populations greater than 300,000/acre and the distribution for the south being of a similar pattern to that for the whole country. However, all the evidence of this and the earlier analyses supports the view that the sowing of oats in the presence of wireworm populations exceeding 600,000/acre is an undesirable risk, and that there is still danger in the range from 300,000 to 600,000/acre.

TABLE 10. *Classification of oats crops and wireworm population*

Wireworm (1000/acre)	Crop result			
	Successful	Poor	Failed	Total
– 300	103	12	3	118
301– 600	31	5	9	45
601–1000	11	7	9	27
1001–	4	6	11	21
All	149	30	32	211

TABLE 11. *Wheat yield and wireworm infestation; south region only*

No. of fields	Wireworm (1000/acre)		Yield (cwt./acre)
	Range	Mean	
8	– 300	146	22·3
11	301– 600	461	17·2
17	601–1000	777	13·3
7	1001–	1816	4·4
43	All	670	15·3

In the south region there were 24 estimations of wheat yields, having a mean of 20·9 cwt./acre, on fields with a mean wireworm population of 494,000/acre. These fields indicated a loss in yield per 100,000/acre increase in infestation of $0{\cdot}59 \pm 0{\cdot}39$ cwt./acre. The rejection of crop failures for wheat produces a greater bias than it did for oats, as nineteen fields are in this way rejected. Adopting the same technique as with oats and including them at half weight, Table 11 is obtained. The loss in yield with increasing infestation is shown in this table just as strikingly as for oats, the rate of loss as computed from the extreme entries being 1·07 cwt./acre for an additional 1000 wireworms/acre. The removal of the bias in the estimation of the regression coefficient thus brings the results for wheat surprisingly close to that for oats.

There were altogether fifty crops of wheat which were classifiable in the same way as for oats in Table 10. The 10-year yield average for 1929–38 was 17·8 cwt./acre; and on this

basis Table 12 was obtained. As is to be expected, this table confirms the indications of Table 11. All the available evidence, therefore, supports the view that loss of wheat yield by wireworm damage was very similar in extent to that of oats.

TABLE 12. *Classification of wheat crops and wireworm populations*

Wireworm (1000/acre)	Crop result: Successful	Poor	Failed	Total
– 300	10	1	—	11
301– 600	6	3	3	12
601–1000	4	5	8	17
1001–	—	2	8	10
All	20	11	19	50

8. PLANT DENSITY AND YIELD

It might be contended that the relationship between plant density and yield had little direct relevance to the problem under discussion. Whether or not this is the case, an examination of this relationship is of interest as throwing further light on the contrasts between regions discussed in §§ 6 and 7. The analysis was made in the same form as in these sections and Table 13 shows the results by regions for 128 fields.

TABLE 13. *Regression of yield on plant density.*

Region	No. of fields	Plants (100,000/acre)	Yield (cwt./acre)	Increase in yield per 100,000 plants	k %
South	45	10·3	19·0	$0{\cdot}83 \pm 0{\cdot}28$	45
West	38	12·5	24·0	$0{\cdot}68 \pm 0{\cdot}36$	35
North	45	12·3	22·3	$-0{\cdot}25 \pm 0{\cdot}23$	−14
All	128	11·7	21·7	$0{\cdot}39 \pm 0{\cdot}17$	21

The last column of Table 13 shows the increase in yield per acre corresponding to an additional 100,000 plants/acre expressed as a percentage of the mean yield of 100,000 plants, and is a measure of the extent to which increased tillering and other competition effects fail to compensate for a decrease in plant density. Table 13 confirms the surmise of the last section that changes in stand in the south, where plant densities were low, had considerable influence on the resulting yield, whereas the high plant densities of the north were little affected by similar changes.

It has been found (Finney, 1941) that for sugar beet and mangolds the value of k for an average stand is about 50 %. There appears to be little evidence on the corresponding competition effects for cereals, though it might be expected that the tillering propensities of the crop would permit a greater degree of compensation for plant loss. The observations discussed here support this view, as only 55 % compensation was found in the south, where plant densities were low. In the north the high plant densities appear to have permitted complete compensation for loss of stand. Indeed in this region there is a suggestion that the stand actually exceeded the optimal, as a result of the very heavy seeding rates employed, though the use of equally high rates in the west was apparently justified. Possibly equally successful crops would have been obtained in the north had slightly lower seeding rates been used.

The result of subdividing the fields of the south region into high and low infestations is shown in Table 14. Again the fields with less than 500,000 larvae/acre show a behaviour

very similar to that of the fields in the north. The separation of the high infestations shows them to have been of more extreme type than the average for the region, the effect of loss of plants on yield having been for them very serious, as 74 % of the proportionate yield of the plants was lost and only 26 % made up by compensating increased yield of the survivors.

TABLE 14. *Plant density and yield; south region only*

	No. of fields	Plants (100,000/acre)	Yield (cwt./acre)	Increase in yield per 100,000 plants	k %
High infestation	22	8·5	14·1	$1{\cdot}23 \pm 0{\cdot}38$	74
Low infestation	23	12·0	23·7	$-0{\cdot}18 \pm 0{\cdot}41$	−9

9. DISCUSSION AND SUMMARY

In the first part of this paper a survey is given of the results of sample determinations of wireworm populations on grassland intended for ploughing prior to cropping in 1940. It is shown that there was a very marked decrease in infestation from south to north of the country. The adequacy of the standard sampling technique employed (twenty samples of cylindrical cores 4 in. diameter) in estimating the population of a field is discussed and tables are given to show the margin of error which may be expected when this technique is applied to grassland. The criticism that an average population per field is not sufficient guide to its actual condition is answered by showing that there are few fields in which there is sufficient irregularity in distribution for there to be any question of portions being cropped differently, and that the number of such fields only slightly exceeds its expectation on an assumption of random distribution.

The effect of wireworm infestation on the crop is considered in the second part of the paper. For oats it appears that in the north of the country wireworm populations were sufficiently low and plant densities sufficiently high to permit a high rate per wireworm of damage to stand. On account of the high plant density, however, competition effects were large and decreases in stand may have been largely compensated by increased tillering of the survivors. The net result was that yield showed little dependence on the degree of infestation. In this region heavy infestations were too few for any test to be made of the natural belief that their effect on the crop would be similar to that of similar infestations elsewhere in the country, and hence sharply contrasted with the effects just described.

In the south, on the other hand, the six provinces had very heavy infestation and also low plant densities. Though the poor stand was probably in part due to the high general level of infestation, the use of low seeding rates was probably a contributory cause at the time when the counts were made. By comparison with the north, the rate of plant loss per wireworm was necessarily reduced because of the greater population to be fed. Nevertheless the plant number was too low for adequate compensation for loss to take place. Consequently the repercussions on yield were large, and there was a decrease of 1·3 cwt./acre for each additional 100,000 wireworm/acre. The average yield of the recorded fields in this region was 6 cwt./acre less than in the north. Detailed examination showed that with infestations below 300,000/acre the yield was very similar to that of the north, but was steadily reduced as the infestation increased, the chance of complete failure having been large in the presence of high populations. The yield also showed a considerable dependence on plant density, the average stand having been so low that the compensation for reductions by means of competition effects was small—on the more heavily infested fields it was smaller than is

normally the case with a root crop. Little need be said of the three provinces which have been called the west region except that in general their behaviour was intermediate between the north and the south.

The evidence relating to wheat is scanty and is confined to the south region. The yields, however, agree remarkably closely with the results for oats, there having been a reduction of about 1·1 cwt./acre for an additional 1000 wireworms/acre. The proportion of crop failures was higher than for oats, but the proportion of heavily infested fields was also higher. It seems reasonable to conclude that, in this part of the country, the attacks on the two crops had very similar effects.

For barley the number of fields available in the records is too small for any analysis of the type discussed to be worth while. The data with regard to root crops are equally scanty. The continuation and extension of the survey during 1941 should help to fill some of the deficiencies of knowledge in respect of crops other than oats. The study of the effects of wireworm in the second season after grass may also be begun when the new season's records become available. For the present the deductions from oats are an interesting, though incomplete aspect of the problem of advisory work on wireworm infestation; in applying them it must be borne in mind that they are based on a single, and in many ways exceptional, harvest year, and that the fields recorded may not have been a fully representative selection of the grassland of the country.

The collection of the material on which this paper is based has been carried out by the Provincial Advisory Entomologists and their staffs. To all these the author wishes to express his gratitude, both for the completion of many complicated sheets of records and for assistance during the analysis in the clarification of obscure points. In particular, thanks are due to Mr E. E. Edwards of the University College of South Wales, Mr R. A. Harper Gray of Durham University, Mr W. E. H. Hodson of Reading University, Mr S. G. Jary of the South Eastern Agricultural College, Mr J. R. W. Jenkins of the University College of Wales, Dr H. W. Miles of Manchester University, Mr H. C. F. Newton of the Harper Adams Agricultural College, Mr F. R. Petherbridge of Cambridge University, Mr A. Roebuck of the Midland Agricultural College, Mr L. N. Staniland of the Seale Hayne Agricultural College, Dr I. Thomas of the University College of North Wales, Mr H. W. Thompson of Leeds University, and Dr C. L. Walton of Bristol University. The author is also greatly indebted to Mr J. C. F. Fryer of the Ministry of Agriculture's Plant Pathological Laboratory for continued advice on the biological aspects of the problem studied and, by no means least, to Dr F. Yates of Rothamsted Experimental Station, with whom originated suggestions for most of the statistical treatment of the data.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

A General Meeting of the Association was held on Friday, 14 February 1941, in the Congregational Hall, Harpenden, the President, Dr H. Martin, in the Chair.

The following papers were read:

I. Recent work on the sheep tick, and its bearing on control measures. By J. MacLeod.
II. Recent work on the sheep maggot problem. By the late R. P. Hobson.
III. Some practical aspects of the sheep blow-fly problem. By I. Thomas.

Summaries of these papers are given below.

I. RECENT WORK ON THE SHEEP TICK, AND ITS BEARING ON CONTROL MEASURES

By J. MacLeod, D.Sc.
Cooper Field Research Station, Little Gaddesden, Herts

The sheep tick, *Ixodes ricinus*, is common in the stock-raising areas of Europe and western Asia; it occurs on the eastern Asiatic coast, and in North America. In the British Isles, it is confined principally to the uncultivated regions—the hills and moorlands, wooded districts or areas of peat bog. Over the greater part of the country the species appears to have two seasons of activity, appearing in large numbers on sheep in spring and early summer, and again, though to a less extent, in the autumn. It is responsible for the transmission of two diseases of sheep, louping ill and tick-borne fever, and is often responsible for widespread pyaemic infections in young lambs. The losses in sheep stock may be very severe if the flock has not been acclimatized to these diseases; stock newly introduced from clean districts may show a death rate of upwards of 50 % during their first season of exposure to infestation by disease-infected ticks.

In considering the possibility of control of this pest, it is well first to consider the factors, biotic and physical, which determine its habits and distribution. It can live on a wide variety of hosts, and it has been shown experimentally that it can survive as a species in the absence of sheep. Temporary removal of sheep stock, for a period of a few years, would not achieve eradication of the tick, although it would doubtless reduce markedly the population level. Striking differences occur in the susceptibility to infestation of sheep according to their health and general condition, so that some measure of relief could perhaps be obtained by improving the general well-being of the flock, by supplementary feeding, and provision of shelter in winter. This would be at most only a supplementary measure.

The physical factor controlling distribution of the tick as a species has been shown to be moisture during summer, with, possibly, temperature during severe winters. The saturated air which it needs for its development on the ground surface and for the survival of the unfed stages in the herbage, is only found, during summer, in rough pasture over peaty or acid soils, where there is a layer of moss and rank or old vegetation. This same blanket of old vegetation protects the tick in winter from the lethal effects of black frosts. These conditions mean that the most suitable areas for tick are precisely those vast expanses of wild moorland and hill whose reclamation is obviously out of the question. Eradication of the tick by improvement of the pastures is thus only feasible in limited areas round the margins of hill country, where ticks have invaded deteriorated permanent pastures as a result of their neglect. By far the greater part of sheep grazings in this country is the untameable rough moor, where the tick cannot be attacked by alteration of its habitat. The only remaining method is to accept the tick as a necessary evil, and to protect the sheep by chemical means.

The activity of ticks as individuals has been shown to be correlated with the air temperature, i.e. the summer phase of relatively low activity, which has been found to occur over most of the country, is a result of the high summer temperatures, and can be trusted to occur, irrespective of whether the

ticks seeking a host in the previous spring had succeeded in feeding or not. This is a rather useful finding, as it means that, provided sheep can be protected during the periods of tick activity, they may be allowed to graze over the infected pastures without precautions for the rest of the year.

Sodium arsenite, when applied at a concentration of 0·2 % As_2O_3, has been found to continue to kill adult female ticks attaching to sheep for a period of 10–14 days. Derris, at a concentration of 0·02 % resin, has a similar effective duration. Coal tar creosote dips confer no appreciable protection against infestation. It has further been found that the addition of wool grease prolongs the period of effectiveness of sodium arsenite, but not of derris. Based on these findings, a protective type of dip has been evolved, containing both arsenic and derris, which protects sheep to a large extent against adult infestation for a period of two or three weeks, the protection during the first week or 10 days being of a very high order because the ticks are killed by derris before they can attach, and so the danger of disease transmission is reduced. For the latter part of the protection period the effect is due to the arsenic, which kills the females after they attach.

For this dip, and especially for derris dips, immersion of sheep for at least a half minute is necessary; experimental evidence, both in the laboratory and field, shows that an immersion period of 15 sec., which is a common interval in practice, results in a very marked reduction of the protection conferred. The anti-tick dip is suitable for protection of lambing flocks, since a dipping immediately prior to lambing gives a tolerable degree of immunity from infestation for the first 2 or 3 weeks of the lambing season, during which time the greater part of the lambing occurs. A second dipping, of as much as possible of the stock, carried out at the end of this period, will usually tide the ewes over until the end of the tick season.

Lambs cannot very well be dipped, but a derris powder dust, which has been evolved at the suggestion of Lyle Stewart, Newcastle University, and tested out on a large scale by him, gives satisfactory protection for about a fortnight.

The harmlessness of derris makes it a useful substance for frequent dipping of sheep where it is desired to reduce a tick infestation by using sheep as trap animals. The method has been used with considerable success on a heavily infested grouse moor. Serial dipping of a flock of year old sheep at fortnightly intervals has been carried out for the last four tick seasons. Mr Walter Moore, Aberdeen College of Agriculture, who is in charge of the experiment, has kindly given permission to use the results obtained to date; these indicate that during the first three years a marked reduction occurred, while last year the infestation, although remaining low, was not much further reduced. It is possible that the population is now approaching a level at which further reduction will be slowed up by the persistence of a large proportion of vermin-supported ticks.

II. RECENT WORK ON THE SHEEP MAGGOT PROBLEM

By the late R. P. Hobson, B.Sc., Ph.D.
University College of North Wales, Bangor

The chief sheep maggot fly is one of the greenbottles, *Lucilia sericata*; for a long time it was thought that this was the only species which infests sheep in Britain, but recent work has shown that other blow-flies sometimes attack sheep. The actual loss due to maggots is very difficult to evaluate in terms of money, as it varies enormously from season to season and with various types of farms. There are two main sources of loss: (1) the direct loss of mutton and lamb due to death or loss of condition; (2) the indirect loss due to the labour involved in looking for infested sheep, dressing these and cleaning up dirty sheep. Deaths mainly occur on hill farms, especially if there is much bracken; struck sheep are inclined to hide in the bracken and thus get overlooked. Since maggots are very liable to occur at the time of hay and corn harvest, this pest may take away labour when it is most wanted.

A great deal of research has been carried out on the question of susceptibility. Weather conditions have a predominant influence, fly attack being most common in moist or muggy weather. The pioneer investigations on susceptibility were carried out in Australia by Seddon and his colleagues; they established that a sheep is only attacked by maggots when in a susceptible condition, moisture and bacterial action being essential factors. Their most important achievement was to show that in Merinos liability to strike around the tail depends on conformation, sheep with skin folds being particularly susceptible. They found that strike on the back was due to a condition which they called 'wool rot'.

In this country, maggots occur most commonly in the tail region, due to the wool being soiled with dung or urine; they also occur quite often in clean wool on the back, occasionally on the flanks. These two types account for about 90 % of the cases. The Australian work on conformation does not apply to British breeds of sheep in which skin folds are absent from the tail region. We tackled the problem of susceptibility at Bangor in a different way, by investigating the conditions required by the sheep maggot. For successful myiasis, (1) the fly must be attracted to lay; (2) the conditions in the fleece must be suitable for the hatching of the eggs and for the growth of the maggots. It was found that various putrefying materials attract sheep maggot flies to lay eggs on live sheep, for example, maggot excreta, incubated urine, loose dung from a scouring sheep. Certain pure chemicals were also found to be attractive, all typical products of putrefaction; indole, skatole, and ammonium carbonate. The oviposition response is specific for gravid females of *L. sericata* and these substances only attract in the presence of live sheep which supply an essential second factor. These results explain the attraction of the fly and the part played by bacterial action; they also provided an excellent method for testing repellents. With regard to the development of maggots in the fleece, moisture proved to be the limiting factor. Blow-fly eggs and larvae cannot survive at the temperature of the sheep's skin unless the relative humidity is at least 90 %. Investigation of the micro-climate at the base of the fleece has shown that the relative humidity is usually below 50 % under dry conditions and seldom exceeds 70 % during rain.

Meanwhile, MacLeod introduced a simple method for producing experimental myiasis, which consisted essentially of placing eggs or young larvae on the skin under a damp plug of cotton-wool. This showed that, for larvae to grow on a sheep, it is only necessary to supply a high humidity, concentrated at a small point.

The majority of natural strikes occur around the tail when the wool has been soiled and this supplies the necessary conditions of bacterial action and moisture. Strike in clean wool over the body can only occur if the fleece humidity is abnormally high; this might result from penetration of the fleece by rain or possibly from excessive secretion of moist yolk from the skin glands. Lennox has shown in Australia that free water reaches the base of the fleece in Merinos during rain; however, investigations by Lennox's technique have shown that penetration by rain is a rare occurrence in Welsh sheep.

Since the development of susceptibility is essential for maggot infestation, one method of control is to prevent this happening. Crutching is a simple way of preventing strike in the tail region. This process consists of shearing wool, preferably with mechanical shears, from the hindquarters and tail so that this region keeps clean. It is an established method in Australia and has given good results in North Wales. The chief difficulty is the prejudice of the shepherds due to the effect on the sheep's appearance. Apart from preventing maggots, crutching saves the shepherd the labour of continually catching and cleaning up dirty sheep.

The alternative method of preventing maggots is by means of chemicals; these may be repellents, antiseptics, ovicides, or larvicides. Advances in this subject have been made possible by the earlier work on the causes of strike; repellents being tested by making sheep attractive with indole or ammonium carbonate, larvicides by the experimental method of producing myiasis. As regards *repellents*, the attraction of sheep for maggot flies is difficult to disguise; so far no repellent has been found which will keep off flies for more than a week or so. Their chief value is probably as surface sprays applied at short intervals. *Antiseptics* are practically valueless against strike around the tail; also, it is extremely difficult to maintain a sufficient amount of antiseptic close to the skin to check bacterial action.

The fly dip which is most generally used is a mixture of arsenic and sulphur which acts mainly through the *larvicidal* effect of the arsenic. About two-thirds of the arsenic in these dips is soluble and Moore has suggested that the soluble arsenic is quickly washed out by rain. However, analysis of different fractions of the fleece showed that leaching by rain is mainly confined to the outer third of the fleece in Welsh sheep. For preventing maggots, it is the arsenic close to the skin which counts and this soon becomes reduced in amount by the growth of new wool. This difficulty might appear insuperable, but a new insecticide, calomel, provides a possible solution. Calomel acts against the eggs and not against the larvae. An *ovicide* appears at first sight an unlikely means of preventing strike as the eggs are laid in compact masses and they hatch in a few hours. However, tests with calomel have shown that this chemical, when mixed with wool, kills *L. sericata* eggs at a distance, the active principle apparently being mercury vapour. Further, if sheep are dipped with calomel, the wool remains ovicidal for a considerable time. In a series of dipping experiments during 1940, calomel at 0·2 % proved as effective as a proprietary arsenic sulphur dip in controlling maggots. Unfortunately the high price of calomel may limit its use as a dip, but it has interesting possibilities as a dust.

A further aspect of dipping is the contamination of the dipping bath. It has long been realized that fly dips become less effective when a number of sheep have been through the bath. The presence of dung and urine in the dip have been blamed for this; recent work indicates that suint, the soluble fraction of wool yolk, may be a more serious contaminant. Suint contains a considerable amount of soap, which dissolves in the dipping fluid and completely changes its physical properties. This results in a decreased retention of dip by the fleece, an increased rate of loss of soluble arsenic from the fleece and a reduction in the period of immunity to artificial maggot infestation.

Finally, there is the question of the treatment of affected sheep. A good dressing should promote rapid healing and protect the wound against restrike. For the latter purpose calomel may prove useful.

III. SOME PRACTICAL ASPECTS OF THE SHEEP BLOW-FLY PROBLEM

By I. Thomas, Ph.D.
University College of North Wales, Bangor

There are two main lines of attack on this difficult problem: to attempt to render the sheep immune or very resistant, or to aim at the extermination or great reduction in the numbers of the pest. With regard to the first of these, one possible method is the breeding of resistant strains, but though there are known differences in the susceptibility of different breeds, the subject has not been pursued in this country. The use of repellents is a possible means of producing artificial resistance which has been investigated by Dr Hobson but has not given very promising results in practice. A line of research not at present taken up is the possibility of inducing resistance in the animal by injection or oral administration of chemicals. The dipping of sheep has received most attention. The dips in use are chiefly larvicides and since the introduction of arsenical preparations for this purpose no great advance has been made; the work on calomel as an ovicide, of which Dr Hobson has given an account, seems, however, to hold out distinct promise, especially perhaps the possibility of using it as a dust. 'Jetting' which has been advocated by Australian workers and by Moore in Scotland has not received the attention in this country that it deserves and the much simpler and eminently practical treatment known as 'crutching' would much reduce losses if farmers could be persuaded to adopt it.

Turning to the question of direct reduction of the fly population, we are at once confronted by the lack of fundamental information about the ecology of the sheep blow-fly. Remarkably little work has been done here on direct trapping and the relations of the fly to carrion. It seems possible that the sheep itself provides the great bulk of the flies, but this is not certain and much more detailed investigation is needed. Further, we do not know what a normal population is and have little information as to the range of flight. Parasites of the fly have received some attention but do not seem to offer a profitable line of work from the practical point of view.

Compared with the advances made by the biochemist, our lack of knowledge of the ecological aspects of the problem is striking and the work of a team of investigators would be justified. There is a gap here that badly needs bridging. Why has so important a subject been comparatively neglected? It is suggested that the chief reasons are: (1) that the farmer regards the sheep blow-fly as a pest that always has been and always will be; he does not realize what it costs him and has not therefore pressed for control measures; (2) that the sheep blow-fly is nobody's job; the Veterinary Investigation Officer tends to regard it as within the province of the entomologist and the Advisory Entomologist has a full time job in dealing with crop pests; (3) lack of funds for ecological investigations; (4) lack of publicity in making the results of research known to the farmer. It is hoped that these conditions may soon be remedied.

REVIEWS

Compendio di Entomologia applicata (*agraria: forestale: medica: veterinaria*). Vol. 1: Part 2*a*. Pp. 449–974. By F. SILVESTRI. Portici: Tipografia Bellavista. 1939.

Shortly before Italy became a belligerent, this part of Prof. Filippo Silvestri's *Handbook of Applied Entomology* was received, the first part having been published in 1934. Prof. Silvestri, who is an Honorary Member of the Association, states in the preface to the whole work that it is his intention to include accounts of all species of insects of importance in agriculture, forestry, medicine and veterinary science that occur in Italy, together with the principal species of Italian Africa and those of special interest in other parts of the world which may be imported. The part under notice deals with some of the Aphididae, the Coccidae and a section of the Anoplura, the previously published part having included the Collembola, Orthoptera, Mallophaga, Thysanoptera and Hemiptera. It is mainly descriptive with brief notes on biology and control measures, an important and specially valuable feature being the large number of careful drawings of the various species, nearly all of which are original. It is much to be hoped that Prof. Silvestri will be able to continue publication of this important work.

C. T. GIMINGHAM

Textbook of General Horticulture. By J. C. SCHILLETTER and H. W. RICHEY. Pp. ix+367. 136 figs. London: McGraw-Hill Publishing Co., Ltd. 1940. 21*s*. net.

This volume has been written for the student requiring a general knowledge of the scientific principles of horticulture, little general biological knowledge is assumed, and a considerable amount of elementary botany and physiology is included. The book is written essentially for conditions in Iowa, U.S.A., and the general treatment of the subject is on good and original lines. In accordance with the common practice in America most plants are referred to by their common rather than their botanical names. It is a little startling to the horticulturist to find in a list of annuals the name 'Babies's breath' (sic) for the plant *Gypsophila elegans*, especially as the perennial species is given its botanical name. The treatment of some of the more complex physiological relationships of plants is so cursory that it would in many cases be better omitted, but in general the book provides a good background for an understanding of the linkage between theory and practice in horticulture.

R. H. STOUGHTON

Plant Physiology. By MEIRION THOMAS. 2nd ed. Pp. xii+596. London: J. and A. Churchill, Ltd. 1940. 21*s*.

The first edition appeared in 1935 (see *Ann. appl. Biol.* 1936, **23**, 201) and was reprinted with slight emendations two years later. In the present edition the general structure remains unchanged but new matter has been added, especially in the chapter on respiration, and in scattered sections dealing with oxidation enzymes, the zymase complex, the absorption of solutes, the translocation and storage of solutes in the cotton plant, mineral nutrition, the composition of chloroplasts, the reactions occurring in photosynthesis, and plant hormones. In spite of condensation and deletion of older matter, the number of pages has increased by 102, and the price of the book by 5*s*. There are four new illustrations, and nearly 100 references are added to the bibliography, but it is a pity that the three partial lists of the two editions and the amended reprint have not been unified: also some of the references cited in the text need attention. Within its restricted field the book is a useful survey of the subject.

W. B. BRIERLEY